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THE PROBLEM OF EQUINE STRANGLES IN INDIA

By F. C. MINETT, Imperial Veterinary Research Institute, Mukteswar.

(Received for publication on 10 December 1943)

(With Plates I and II and three text-figures)

THE object of this article is to place on record and to discuss figures relating to the incidence and mortality from strangles which have been collected over a number of years from Army Remount Depots and Stud Farms in India by the Army Veterinary Department and by the Imperial Veterinary Research Institute. The opportunity will also be taken to set out the procedures that have been commonly adopted at depots and farms in maintaining remount stock.

The term 'strangles', as used in this article and as sometimes used in a clinical sense, is a comprehensive one and may be taken to include not only cases with the typical sub-maxillary abscess, associated or not with suppurations in other parts of the body, but also cases of acute nasal catarrh or laryngitis in young horses, especially where there has been contact with typical abscess cases. Certainly in practice, the definition of 'strangles' is an elastic one and will no doubt vary according to the fancy of different veterinary officers, but from our present point of view clinical niceties matter little. What we are now concerned with is a more or less acute affection involving the upper respiratory tract of young horses or mules frequently accompanied by suppuration of the sub-maxillary lymph glands or other glands about the throat, occasionally followed by pyaemic lesions in other regions of the body, occasionally fatal and always to be regarded as infections. From recent researches, there can no longer be reasonable doubt that this disease complex is caused by hæmolytic streptococci, belonging to Lancefield's serological group C, though not necessarily of the same serological type [Bazeley, 1942].

BREEDING OF HORSES AND MULES FOR ARMY PURPOSES AND THE PROCEDURE ORDINARILY ADOPTED AT ARMY REMOUNT DEPOTS AND STUD FARMS

The Director, Army Remount Department, has been good enough to provide the following information. "Between 1927 and 1939 the number of 'branded' mares under control of the Remount Department in India averaged 12,696 in each year. The main breeding operations were carried out in the Civil Districts of Lyallpur, Sheikhupura, Jhang, Shahpur, Montgomery and Multan. 'Unbound' horse-breeding (now abol-

ished) was also undertaken during the same period in Rawalpindi, Attock, Gujarat and Hazara districts. Young stock are purchased by officers of the Department from September to March each year and are sent to the Army Remount Depots at Mona and Sargodha in the north-west Punjab. During this purchasing season the officer tours his area three or four times. The procedure in the case of these 'area-bred' animals is that all young stock are sent by road, a distance up to say ten miles, to collecting centres for inspection by the Remount Officer. Animals stay in the collecting centres for periods not exceeding 24 hours, and, if purchased, they are then trucked in lots of eight or ten and sent by rail to the depots, the journey taking anything up to 24 hours. The normal age of purchase is 12-18 months, but in the case of well-developed or backward stock these general limits may be varied at discretion. Also, mules may be bought when only six months old. With horse stock the average purchase age has been fixed at 12-18 months, since experience has shown that animals less than 12 months old develop more severe strangles than older ones."

Remount Depots

To give a preliminary idea of the magnitude of the strangles problem, it may be said that Mona and Sargodha depots each cover some 10,000 acres of land and that at any one time each of them may have to accommodate up to 2,000 horses and as many mules. The land is flat and canal-irrigated and grows such fodder as lucerne, *shafal*, berseem, *jowar*, oats, barley, gram. The general principles of management at both depots are the same. The young stock on arrival are grouped in 'dry' reception paddocks (i.e. without natural grazing) in a part of the depot set aside as a 'segregation area', in which the strangles hospital are situated. In this area they remain until the close of the 'strangles season' in May, when they are moved into the depot proper, the reception paddocks then remaining free of stock until the next purchasing season. In the depot proper the animals remain until they are issued to military units at four years of age (mules) or five years (horses). Feeding throughout is liberal, consisting of green fodder grazing, oat hay, *jowar*, barley and other concentrates.

Mona

Segregation. At this depot the segregation area covers about 200 acres and is separated from the depot proper by a railway track. As the young stock are received, they are grouped in lots of 10-15 and are kept for the first three days in mud-wall enclosures (Plate I, fig. 1), containing a *chappar* or shelter and situated within the reception paddock which itself covers about two acres. The object of this incarceration is that the animals may get thoroughly used to one another and quieten down. After this, they are allowed into the paddock to 'get used to wire'. If, as sometimes happens, paddocks with walled enclosures are not available, the animals are turned into paddocks which have simply a central railled-off *chappar*, this being a more open arrangement. Each unit of 10-15 animals is kept entirely separate from other similar units, e.g., they go separately for grazing. Most of the young stock horses bought are colts, because (a) they are more useful for army work, and (b) the fillies are mostly required for replacement purposes in the breeding area. The fact, however, that some fillies are bought causes some difficulty in distribution while in segregation, since they have to be kept separate from the colts. It may finally be noted that, when owing to depletion in numbers by strangles not more than three or four animals are left in a reception paddock, the remainder may be mixed to form a fresh unit.

As soon as an animal develops a typical attack of strangles, it is removed to a hospital set apart for this disease. For the more severe cases, the accommodation is of the 'open-stall' type, the stalls being about ten feet square, with earth floors and iron roofing (Plate I, fig. 2). Mild cases may have to run loose, owing to shortage of stall accommodation (Plate I, fig. 3). On discharge, the animals go to grazing convalescent paddocks, specially allotted for such cases, each paddock being wire-fenced and about two acres in extent, intended for ten animals and containing a 'dry' enclosure for feeding and sheltering at night (Plate I, fig. 4). Animals discharged from hospital do not go back to their original reception paddocks but remain in the convalescent paddocks until May. Any animal that may happen to develop strangles after transfer to the depot proper is likewise treated in the strangles hospital. It may also be noted that simple catarrh or fever cases may be treated not in hospital but under isolation in the convalescent paddocks.

It is understood that up to about 1927 the segregation procedure at Mona was essentially the same. The segregation camp was located in a more or less confined area and consisted of 30 paddocks. In 1927 the paddocks were changed

from an unsatisfactory 'inter-communicating' type to a 'multiple dispersion' type which aimed at reducing the chances of contact among the young stock. At the same time, there was introduced a secondary segregation or 'semi-segregation' for stock which had recovered from strangles two or three months before. For this purpose, areas consisting of both dry and grazing paddocks were used, an excellent arrangement for convalescent animals. Since 1931, the amount of grazing has been increased, and this is now provided for all incoming young stock as soon as possible after their arrival.

Post-segregation. The system, which is at present in operation and which is similar in principle to that adopted in earlier years, is in outline as follows: Young stock horses coming out of segregation are first moved into a certain area of the depot proper where they remain for 12 months, assembled in groups of up to 40 animals. At the beginning of the next year, by which time they are two and a half to three years old, they are moved to another part of the same area into paddocks capable of holding as many as 50 or 60 animals (Plate I, fig. 5). (There are now some 35 paddocks of this type in the primary post-segregation area at Mona). At this time they are castrated, and they remain in this area until they are approximately four years old. The paddocks here are dry ones and are often separated by a single fence of wire. At the centre of each is a square mud-brick enclosure (for food, etc., storage), provided on the outer side with mangers and usually situated near trees for shade. The size of the paddocks is variable, from 6 or 8 to 15 acres, and close by are grazing lands (berseem, lucerne) where the horses are allowed to remain for several hours daily. At night they remain free in the dry paddocks. After the animals have reached four years of age, they are brought into the 'Home Lines' where they are given some training (riding, lunging) prior to issue. By this system of movement horses purchased in one year are kept separate from those of another year.

The above account refers to young stock horses. Mules purchased as above in the breeding areas are treated similarly, but they are kept in larger groups during their period in segregation, partly for administrative reasons and partly because they are more hardy. After their first year they are moved into the depot proper where they remain till two-and-a-half to three years old; they are then distributed anywhere in the depot proper for work until they are issued.

argotha

The situation at this depot has been similar on the whole to that at Mona, and it is only necessary to call attention to certain differences.

Segregation. In 1924 there were three main segregation blocks, known as A, B and C, each quite separate from the other and in fact one to two miles apart. Each block was enclosed by wire and was divided up by wire fencing into small paddocks. 'A' block, for example, had some 16 dry paddocks, each of about three to four acres, and into one of these as many as 20-40 fresh purchases might be placed. Each block had its own strangles hospital and convalescent paddock where animals were put for one to two months prior to removal to another part of the segregation area. Major W. N. Rowston, R. A. V. C., has stated that in 1924 young stock horses on arrival were first put into block A and, when this was full, blocks B and C were brought into occupation. At a later date, it appears that block C was reserved for mules. It is also stated that, when the numbers of horses in a paddock were depleted by strangles, it was the practice to fill up with young mules. It is recounted that some of the reception paddocks had a feeding *chappar*, while others had none, feed-troughs made of dried mud being placed around the bases of trees. Actually, there is now little shelter in these paddocks, as many trees have been injured by waterlogging of the soil. At the present time, 'A' block alone remains, while other arrangements have been made for the segregation of mules. Plate II, figs. 6 and 7 show the type of reception paddock and hospital accommodation at present existing.

Stud Farms

Information on the subject of this paper has also been received from four Stud Farms, partly maintained for Army Remount purposes, viz. those at Probhynabad, Coleyana and Renala in the Punjab and that at Ahmednagar in Bombay. In passing, it should be made clear that the rearing of horses for army purposes is not confined to stud farms, that is farms managed under the Horse-breeding Grantee Scheme. Considerable numbers of horses are also produced in villages of the Punjab under an 'area-breeding' scheme, where people may keep only one or two mares. On the other hand, some of the stud farms are of considerable size. Coleyana and Renala Estates, for instance, having about 7,500 acres of canal-irrigated land and maintaining some 170-200 brood mares and 100-150 young stock (birth to four to five years), in addition to thorough-bred stock which are raised under private enterprise. Under the conditions of the horse-breeding grant, brood mares are approved by the Army Remount Department, are mated with stallions similarly approved, and the offspring offered at fixed prices to the Army from 12-18 months of age. If accepted, they are transferred to Mona or

Sargodha, otherwise they are sold privately or in the case of suitable fillies retained for breeding purposes. At Ahmednagar, which was closed in 1940, it was the practice to keep selected stock to four to five years old and then send them direct to units. Table IV shows the distribution of young stock by age groups for two of the farms, Probhynabad and Coleyana. At the former stud those in the age groups of one, two and three years are in roughly the same proportion, while at Coleyana the proportion of yearlings is higher than at later ages.

The animal husbandry procedures at the different stud farms no doubt vary in detail according to the will of the owner. According to the general plan, mares foal chiefly in February to June and smaller number in September to November, foals are weaned at six or seven months, drafted into batches of 10-15 and after some time the sexes are separated. Stock are turned into grazing (*shafai*, lucerne) or exercise paddocks during the day time and are sometimes brought into small enclosures at night. On the Estates the agricultural unit is the 'square' or 'rectangle', which covers 25 acres. At Renala there are 44 such squares, and most of these are subdivided into four six-acre paddocks for rotational grazing, with a centrally situated dry paddock with feed *chappar* and trees for shelter (Plate II, fig. 8). Each such square accommodates 10-12 animals. At Coleyana, the stud area is divided into paddocks intended for exercise, grazing or for growing fodder crops. This area is changed over about once in five years, and a similar area which has been under crops is taken over for the stud. At present, the squares are being step by step divided up into paddocks of five acres each for rotational grazing or for purposes of exercise. An open-air system is adopted on this estate, the mares foal in the open, and as a general rule the stock remain out at night, with trees and hedges as the sole protection. The only exception to this is that, after weaning, the animals are kept in half-acre mud-wall enclosures (Plate II, fig. 9) for about a month, so that they may be broken to handling. Stocks are also brought into the yards twice daily for grain feeding and rough grooming. Watering is from troughs connected with small tube-wells. For hot weather, ponds are provided for stock to cool themselves.

CASE INCIDENCE OF STRANGLES BY YEARS

Remount Depots. Up to 1933, the figures available are for financial years (April to March), and subsequently for 'strangles years' (September to August). It would have been difficult to bring the figures on to a common basis, but for a

broad consideration of the incidence or attack rate by years, the labour involved in making the adjustment would scarcely have been warranted. The data for horses at Mona and Sargodha are recorded in Table I, and for mules at these two depots in Table II.

From the frequency distribution shown in Table I, it is seen that at both depots the annual attack rate has only once fallen below 50 per cent. and that in some years practically all have shown clinical symptoms. Among horses at Mona there are indications that 1920, 1925, 1929, 1933 and 1939 were peak years, while at Sargodha the yearly distribution is more uniform. Data are not available to show whether there is any variation in the susceptibility of young stock, since the number of animals at risk at different age groups is unknown. At Mona the mean annual attack rate over 23 years (1917-40) has been 76.2 per cent, while at Sargodha over 13 years (1927-40) it was 72.6 per cent. By applying the χ^2 test, it can be shown that since 1927 the attack rate in horses at Mona has usually been higher than at Sargodha, and the same is true since 1929 in the case of mules.

In young stock mules (Table II) the incidence of strangles is definitely lower at both Mona and Sargodha than among horses, the mean annual attack rates being 53.2 and 46.8 per cent respectively. The yearly distribution is fairly uniform.

Stud Farms. The information is shown in Table III. The first point is that the incidence is very variable from year to year. In some years strangles is absent, or nearly so, while in others the rate reaches 50 per cent or even higher. Thus at Probynabad, 1914, 1918, 1924, 1928 and 1933 are peak years, and at Coleyana the same is true of 1930, 1935 and 1937*. There are indications then both from the depots and stud farms that a high strangles morbidity rate has occurred with some regularity every four or five years, and that on the farms in intermediate years the rate may fall very low indeed. The latter feature is in contrast with the more uniformly high rate at the Remount Depots. No attempt has been made to calculate χ^2 values for the stud farms, since the figures are small and there are few overlapping years in which a comparison can be made.

CASE INCIDENCE OF STRANGLES BY MONTHS

Full details of the position under this heading are reserved for a paper in which the seasonal incidence of strangles is considered. At some risk of anticipation, however, Fig. 2, based on the data, is introduced to show that at the depots, as

the strangles year progresses the attack rate rises to the month of April and then rapidly declines. As between Mona and Sargodha during the period 1933-40, there is little difference in the date of onset of the disease, but from 1934 on cases continued to appear rather later at Mona than at Sargodha. The influx of new purchases continues during the period September to March, after which the population remains stationary (Fig. 1). From Figs. 1 and 2, it would appear that the gradually increasing incidence from September onwards is connected in some way with the increasing number of susceptible stock within the area.

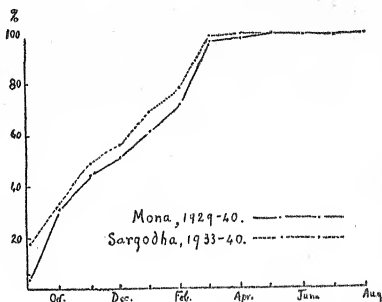


FIG. 1. Young stock in segregation (horses), total monthly population in segregation as per cent of total population during period.

Total young stock segregation at Mona, 1929-40-6525

Sargodha, 1933-40-4075

As to the stud farms, Fig. 2, prepared from data for Coleyana and Renala, shows high points from December to February, a secondary rise in June and a decline through July and August. This curve, it will be observed, is different from that for the depots.

MORTALITY RATES

Horses. At Mona the death rate has varied over the period of years from 0.0 to 8.5 per cent of animals affected, mean 5.0 (Table I). At Sargodha, the figures for the shorter period are rather higher, viz. 3.5 to 10.1, mean 6.1 per cent and the χ^2 test shows that in certain years the differences from Mona are significant. At the stud farms (Table III) the death rate, calculated on 2123 cases of strangles, was 6.1 per cent, a figure similar to that of the remount depots.

The mortality rates over the series of years at Mona and Sargodha have been analysed to see if any significant variation exists in different years.

*1943 at Coleyana was also a peak year.

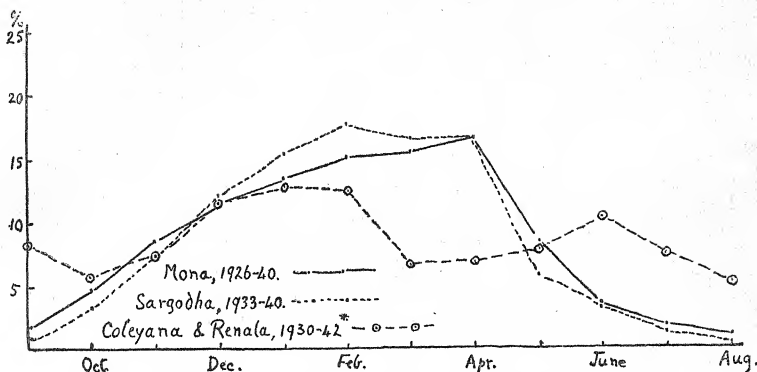


FIG. 2. Case incidence (horses).
 Total monthly incidence as per cent of total incidence during period.
 Total incidence at Mona, 1926-40=8437
 " " " Sargodha, 1933-40=3093
 " " " Coleyana and Renala, 1930-42*=874
 * Period of 21 years.

For this purpose mortality rates have been placed in groups, in each of which there was a small difference only in the rates. Thus for Mona, four groups have been formed with mortality rates of 7.2, 4.8, 3.0 and 0.9 per cent; at

Sargodha, three groups with rates of 8.8, 6.2 and 3.7 per cent can be formed. Thus both at Mona and Sargodha, definite differences in mortality rates exist, confirming that strangles does vary in its 'severity' in different years.

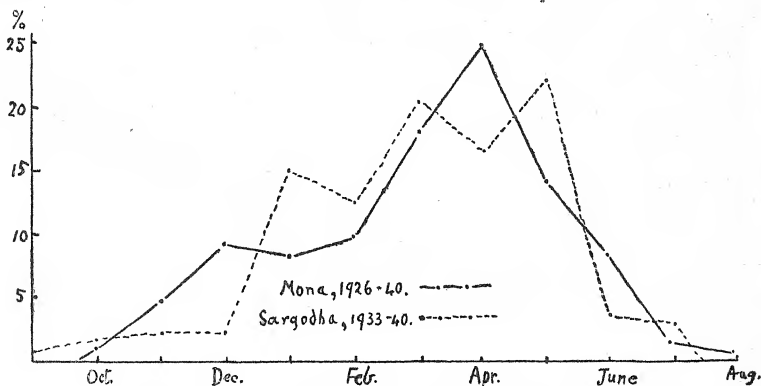


FIG. 3. Case mortality (horses).
 Total monthly mortality as per cent of total mortality during period.
 Total deaths at Mona, 1926-40=298
 " " " Sargodha, 1933-40=190

Fig. 3 shows that at the depots the proportion of fatal cases rises as the strangles season advances, which conforms to the suggestion that attacks later in the season are more severe. At the stud farms the rates do not appear to differ greatly throughout the year.

As to the ages at death, figures for 27 animals are available for Coleyana.

Age in months . . . 3 6 9 12 15 18 Over 18
Number dying . . . 0 1 4 8 8 3 3
Deaths are, therefore, most frequent in yearlings.

Age (months)	3	6	9	12	15	18	Over 18
Number	11	83	86	123	5	50	
Per cent							—81.6—

The proportion of older animals admitted is perhaps surprisingly high. Of the younger ones, the largest proportion fall in the age groups 10-12 months, the next largest proportions being four to six and seven to nine months. A study of the outbreaks coming within the period shows that the bulk of the earliest clinical attacks appears in younger animals, though it is of course possible that infection may have been acquired in the first instance from adult carriers.

DISCUSSION

Opinions and experiences of earlier observers on strangles. Many years' experience has taught veterinary officers that strangles cannot be prevented from appearing in Army Remount Depots, and many believe that the main object should be to produce a mild form of the disease in early life, which will leave a long and serviceable immunity. Unfortunately, while strangles is always of most importance during the first season the young stock are in the depot, the disease is said to vary greatly in its incidence and severity from one season to another and from one outbreak to another; and this has not only caused difficulties in attempts at natural immunization but has also made it difficult to judge the efficacy of methods of prevention. It has been noted, however, that at the depots during any one season the prevalence and severity of the disease increase with the passage of time to reach its highest intensity in March and April, irrespective of the date of onset. Capt. G. W. Short [1939] has also remarked that the first cases in a season are mild and succeeding ones tend to be progressively more severe. It has, therefore, been recommended that young stock should be purchased as early in the season as possible and never later than March. On the question of immunity, mules have a considerable natural resistance, and in them strangles is mild and death rare. In horses, while second attacks are comparatively rare, there are numbers of reports (e.g. Langley, 1938; Short, 1939) of a second attack in the same animal one to eight

Mules. The mortality in mules (Table II) is much less than in horses, being about 1.0 per cent. There is no significant difference as between Mona and Sargodha, nor in mortality rates over years.

AGES OF ANIMALS AT ADMISSION

The following figures for 543 cases at Coleyana over the period 1930-43 show the age groups of the animals on admission for strangles.

Age (months)	3	6	9	12	15	18	Over 18
Number	11	83	86	123	5	50	
Per cent							—81.6—

months later, the first attack having sometime occurred before admission to the depot. As to the time elapsing between exposure and appearance of symptoms, while some young horses may show symptoms within a few days of arriving at the depot, Edwards [1926] has commented on the fact that many cases crop up some time after admission.

As to the phase of maximal infectivity of cases the belief has been expressed that animals are most dangerous spreaders during the early stages of the attack, while on the question of spread from recovered animals opinion is divided. As to the influence of weather conditions, a subject to be dealt with in another paper—it has been remarked that heavy rains are liable to be followed by a number of cases, while in mild weather the disease tends to be less severe.

Efforts at control. A great deal of attention has been paid to this problem at Mona and Sargodha, but without striking success. Reports of this work are given by J. T. Edwards [1923-25, 1925] and Arnold [1932]. The measures tried, apart from those designed to inhibit the spread of infection, have included the use of streptococcus vaccines of various kinds (dead cultures; living cultures of low or high virulence; sensitized, formalinized or 'extracted' cultures; 'pyovaccine'), convalescent serum with exposure to natural infection, serum combined with the nasal application of infective material, as well as various proprietary preparations. As curative agents, such drugs as trypanamide, sulphonamide P, prontosil, etc., have been tried. During the past ten years the tendency has been to recognize that useful results were not to be expected from such methods and to rely on measures for preventing the spread of the disease or producing mild attacks. Means which may be adopted with this end in view may now be considered. Brigadier A. J. Williams [1932] considered that a fair test of the efficacy of preventive measures would be the percentage of young stock admitted during the year which develop the disease, while

the efficacy of curative measures may be gauged in part by the case mortality. He has pointed out that factors having a bearing on the result of preventive efforts would be the total number of young stock admitted during the season, the accommodation available for them, and the number infected before admission. As regards curative measures, the mortality rate should not be the sole criterion and account should also be taken of the duration of illness as determining the setback to normal development. Judged by these criteria, he concludes that up to 1932 the measures adopted at Mona and Sargodha have been unsuccessful.

On the question of control we may record the following more important recommendations in a report dated 1923 by Major H. Allen:

(1) the Veterinary Officer should be in absolute charge of segregation;

(2) young stock should be bought and admitted as early in the season as possible and in large batches. Batches of young stock admitted should be separated as far as feasible from previous batches;

(3) young stock should not be purchased while showing symptoms;

(4) when strangles appears in animals of a particular block, fill up with young stock mules;

(5) never overcrowd the paddocks or the segregation area. Not more than 12 to 15 animals should be accommodated in each paddock. Paddocks should also be well isolated from one another;

(6) do not attempt to 'classify' stock while in segregation, as this means altering the constitution of groups;

(7) catarrh cases may be treated in their paddocks, but typical strangles cases should be immediately removed to strangles paddocks and severe cases to strangles hospitals;

(8) keep young stock in segregation for not less than three or four months;

(9) provide for a convalescent paddock in the segregation area;

(10) after passing through segregation, stock should be accommodated for a month in 'semi-segregation';

(11) stock should be protected from chills; and

(12) cleanliness and disinfection are important. Manure should not accumulate. Soiled fodder and litter should be burnt. Disinfection of buckets, water troughs, twitches, and the hands of attendants.

Fig. 1 suggests that the whole of recommendation No. 2 has not been observed, but with the possible further exception of No. 11, all other recommendations, we believe, are being satisfied. We would, however, add, as recommendation No. 13, a satisfactory food and water supply.

Epizootiology of strangles. As a problem in epizootiology, that of strangles at the remount depots appears to be a fairly straightforward one and can be dealt with on lines which are familiar to students of the subject. The rise in the incidence of strangles when young horses are brought together somewhat resembles the epidemic rise of naso-pharyngeal infections which follow the reaggregation of children shortly after the commencement of the school terms. It may be a useful lesson to try to visualize for a moment what may really be happening when a batch of 30-60 young horses is brought in and then split up into smaller groups of say 12 to 15 each. At the time of arrival, the animals are certainly not normal in the sense that all are free from the streptococci which cause strangles nor as possessing a uniform susceptibility to these streptococci, should they chance to meet them. We may assume that some, perhaps many of them, although apparently in good health, will be harbouring the specific streptococci in some part of the upper respiratory tract (healthy carriers or latent cases), a few may be suffering from atypical strangles in the form of nasal catarrh, and the rest will be really healthy. It may next be noted (a) that the status of many animals of the group, in respect of their bodily resistance and of any pathogenic streptococci they may harbour, has changed since their earlier days in the breeding areas, because they are now older, may already have experienced strangles, have mixed with new companions, and have just undergone the fatigue of a journey to the depot; and (b) that their status is destined to change still more profoundly under the new conditions they will experience at the depot. Thus it may be expected that, as a result of the unusually close contact before and during the journey as well as of the new grouping at the depot, the carrier rate will rise, and in due course clinical cases of strangles will be liable to appear, in which case increasing amounts of infective material will become available to other animals of the group. The closer the contact the more rapidly will spread take place and the larger the dosage of infective material likely to be absorbed. The final result will be that all the animals will quickly become infected and react in various ways, with the possible exception of those which have secured a substantial measure of immunity from attacks of strangles in the breeding areas. Thus, towards the close of the purchasing season a horse population will be established having a level of naturally acquired immunity which is fairly high on the average and which is not likely to be disturbed by the specific streptococci still existent among them or by any further happenings. Unfortunately, in the past

this result has only been attained at the price of much acute sickness and possibly severe setbacks in bodily condition. Fortunately, on the other hand, the population will not have been much depleted by death, since strangles is not a killing disease. The misfortunes that may befall our batch of 60 horses will perhaps be aggravated by the arrival at intervals of similar batches, which, although distributed in other paddocks, are still lodged within the area set apart for segregation.

We may now add some comment on the above hypothesis. The suggestion that most of the animals in a group will soon become infected is supported by the history of events at Mona and Sargodha where the annual attack rate has tended to be so high. Incidentally, by analogy with what is known of scarlet fever a seasonal rise in the carrier rate would be expected in the autumn and winter months.

It has frequently been said that the increasing prevalence and severity of cases during an epidemic may be due to the increasing infectivity and virulence of the parasite during its passage from host to host. There is, indeed, some evidence for the existence of special epidemic strains in some streptococcus infections of man. These are strains which are endowed with a highly-developed power of spreading among susceptible people and of killing them. There is also some evidence of the sudden appearance of hypervirulent strains in the case of normally slow-spreading streptococcus epidemics in small animals. In the case of strangles also some increase in infectivity (invasiveness), together with some increased power of causing tissue damage, cannot be ruled out but on that point there is no information. However, environmental factors acting upon the host, as well as the simple congregation of infected and susceptible animals is well known to be sufficient by itself to enable epidemics to progress, so that it is unnecessary to look for special properties in the parasite.

It has been suggested above that the streptococcus of strangles in different animals may produce a healthy carrier state, a simple nasal catarrh or typical strangles. Possibly, ideas regarding this are now changing, but we may note in the first place the analogous cases of 'snuffles' in rabbits. 'Snuffles' is the clinical term for a common disease condition of rabbits, caused by a pasteurilla and associated with pus in the nasal sinuses, and accompanied by nasal discharge and sneezing. Webster [1924] has shown how healthy rabbits, i.e. rabbits free from pasteurilla, vary in their response to the nasal instillations of the same amounts of living cultures of the organism. Such rabbits may show (a) no response, (b) a

'healthy' carrier state, (c) 'snuffles' or (d) pneumonia or death, the result of the experiment in the individual animal depending on its resistance. Since we are dealing here with a respiratory affection, the analogy to strangles is apt. Still more apt is the case of outbreaks of respiratory streptococcus disease in man where the same infecting agent may produce (a) a healthy carrier state, (b) a feverish catarrh, (c) tonsillitis of varying severity, and (d) scarlet fever. Recent researches on equine affections in Australia by Bazeley and Battle [1940] have provided some direct proof in showing that at least some of the streptococcus strains from simple catarrh are identical by agglutination and other tests with those from strangles. They isolated 457 strains of haemolytic streptococci from 415 cases of disease in horses. All these belonged to serological group C of Lancefield and by agglutination could be split into five main types. Although the authors find that the specific types of equine streptococci do not always appear to be associated with specific diseases, it may be noted that all the strains from fresh strangles abscesses—45 in number—and 58 of 200 strains from pure respiratory catarrh cases belonged to the same agglutinative types. Incidentally, it may be observed that a study of the factors involved in the spread of 'snuffles' and of lymphadenitis in guinea-pigs—a strangles-like disease which occurs naturally in these animals and caused by group C streptococci [Seastone, 1939]—might provide valuable data in connection with strangles in horses.

Control of strangles. The final point to be considered is whether any particular steps can be taken to reduce the incidence of strangles in the depots or to reduce the severity of cases.

In the case of our particular problem, there are two fundamental considerations: firstly, that it is not a question of trying to eliminate the disease altogether, and, secondly, that any practicable means of immunization must await the outcome of academic research.*

Here it may be pointed out that the problem of streptococcus disease in human beings is recog-

*Recent work appears to have brought this desideratum nearer. While it has been shown, for instance by Stamp [1936], that some degree of type-specific immunity against haemolytic streptococci can be produced in rabbits by the use of broth cultures killed by heat, Loewenthal [1938] has pointed out that very young cultures, which consist mainly of encapsulated organisms, ought to be used and that the heat for killing them must be very cautiously applied. Bazeley [1940, 1942] in Australia has now proved that by similar methods a vaccine can be prepared from strangles streptococci. He has shown that this vaccine is capable of affording some immunity to mice, that a protective serum can be prepared by its use in rabbits, and that it has definite immunizing power in horses. The possibilities of successful immunization are thus distinctly promising.

nized to be an enormous one and is even regarded by some as insurmountable. It is not likely to be a smaller problem in communities of young horses, unless they are subjected to a good deal of restraint. Such restraint must be on epidemiological lines, and aimed at promoting as effectively as possible the dispersal of young stock from the moment they are admitted. During recent years the truth of this has been well recognized by the veterinary officers concerned. We do not believe that at the present time there is overcrowding of the depot paddocks, or that it is practicable to disperse stock more than is being done now. To emphasize the point, however, we would refer to the case of experimental epidemics in mice, from which valuable lessons may be drawn. Topley [1922, 1926] has shown that when mice, which had been exposed to the risk of infection with *Bact. typhi-murium* and among which an epidemic was imminent, are dispersed into groups containing 19 to 25 mice, there is little effect on the mortality, but when they are dispersed into smaller groups of ten animals each, the mortality rate is greatly reduced. Once the epidemic has started, however, dispersal into still smaller groups of five mice each had a much less marked effect. Topley, therefore, suggests that, if at the first sign of an outbreak animals are split into small groups and after a certain interval are brought together into groups of more manageable size, a severe outbreak may be forestalled. The importance of adequate spatial distribution of subjects at risk is also admirably shown by the observations of Glover [1918] on cerebrospinal fever in a military unit. He found that the meningococcus carrier rate provided an index of whether there was overcrowding in the sleeping quarters. A carrier rate reaching a certain figure, 20 per cent, could be taken as a danger signal. In order to reduce the rate and avert the danger of an outbreak of cerebrospinal fever, it was merely necessary to increase the distance between the beds.

Always to be borne in mind in the case of strangles, as of other infectious diseases, is the question of whether the movement and distribution of young stock can be so changed as to lessen the frequency of contact between them. Admittedly, the question is more easily asked than answered, but as Topley [1942] has said 'Any step that lessens the probability of effective contact, direct or indirect, is a step in the right direction. As we reduce the frequency of effective contact, we reduce the mass of infective material on which the probability of further diffusion in part depends.'

The earlier in the purchasing season young stock can be brought into the depots the better, in this way a process of natural immunization arising from intimate association in the paddocks

will have a longer time to develop before the cold spell of winter. But since natural immunization is largely an affair of chance, it is unlikely to be so satisfactory as an efficient specific immunization, so our hope for the future lies in the latter, assisted by good dispersal and protection from adverse weather conditions. As to the isolation of declared cases of strangles in hospital, this is not likely to do much good so far as the animals which remain behind are concerned, especially if we may assume that these include carriers and that carriers and atypical cases have at least some importance as spreaders. At the same time, any systematic attempt at isolating infected animals other than declared cases would be very difficult under conditions at the depots. Finally, it may be observed that one way of by-passing the problem would be to replace young stock horses largely by young stock mules, since these have considerable inborn resistance to strangles.

As to the treatment of clinical cases, sulphanilamide therapy is worthy of more extended trial, especially for cases in the very early stage [Minett and Edwards,* 1945.]

SUMMARY

1. The article is concerned with strangles as it has occurred in two large remount depots and on certain horse-breeding farms in the Punjab. The annual attack rates and mortality figures are given for various periods between 1914 and 1942.

2. Some account is given of the methods ordinarily practised at these remount depots and stud farms in dealing with young horse populations.

3. The principles involved in control of the disease in remount depots are discussed.

ACKNOWLEDGEMENTS

Information on horse-breeding in India has been kindly provided by the Director, Army Remount Department. I am indebted to Brigadier J. J. M. Soutar, C.B.E., Director of Army Veterinary Services in India, for his interest and support and to the officers named in the text for their recorded opinions of which full use has been made. Considerable help has also been obtained from the officers in charge of the remount depots and owners of the stud farms. Special mention is to be made of the officer in command at Mona, Colonel M. F. Keightley, A. R. D., of Veterinary Officers at Mona and Sargodha in 1943 (Major P. F. Woodland and Capt. G. McElligott, R. A. V. C.); of Major W. P. S. Edwards, R. A. V. C., and of Lieut. D. H. Witherington, R. A. V. C., acting Veterinary Officer at Sargodha in 1943. Table I is partly based on a chart prepared by Capt.

* In press.

D. I. C. Tenant, R. A. V. C. The statistical calculations are the work of Mr. S. Sen, Statistician to the Institute.

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TABLE I
 Incidence of strangles in horses at Remount Depots by years

Year	Mona			Sargodha		
	Young stock received	Affected	Case mortality	Young stock received	Affected	Case mortality
1917-18 . . .	232	143 (61.6)	6 (4.2)
1918-19 . . .	168	85 (50.6)	2 (2.4)
1919-20 . . .	223	174 (78.0)	5 (2.9)
1920-21 . . .	152	150 (98.7)	0 (0.0)
1921-22 . . .	203	112 (55.2)	3 (2.7)
1922-23 . . .	397	243 (61.2)	20 (8.2)
1923-24 . . .	451	364 (80.7)	31 (8.5)
1924-25 . . .	619	622 (84.3)	39 (7.5)
1925-26 . . .	558	556 (99.7)	37 (6.7)
1926-27 . . .	874	340 (38.9)	7 (2.1)
1927-28 . . .	511	332 (65.0)	17 (5.1)	379	242 (63.9)	14 (5.8)
1928-29 . . .	593	350 (59.0)	11 (3.1)	426	348 (81.7)	19 (5.5)
1929-30 . . .	342	327 (95.6)	4 (1.2)	455	369 (81.1)	29 (7.9)
1930-31 . . .	617	502 (81.4)	16 (3.2)	441	279 (63.3)	15 (5.4)
1931-32 . . .	530	424 (80.0)	17 (4.0)	469	265 (56.5)	10 (3.8)
1932-33 . . .	629	570 (90.6)	22 (3.9)	449	262 (58.4)	21 (8.0)
1933-34 . . .	595	575 (96.6)	34 (5.9)	505	337 (66.7)	25 (7.4)
1934-35 . . .	657	557 (84.8)	37 (6.7)	601	535 (89.0)	21 (3.9)
1935-36 . . .	793	598 (75.4)	27 (4.5)	714	481 (67.4)	17 (3.5)
1936-37 . . .	830	596 (71.8)	36 (6.1)	650	450 (69.2)	37 (8.2)
1937-38 . . .	671	544 (81.1)	45 (8.3)	674	467 (69.3)	30 (6.4)
1938-39 . . .	466	325 (69.8)	7 (2.2)	407	338 (83.0)	34 (10.1)
1939-40 . . .	395	383 (97.0)	18 (4.7)	524	490 (93.5)	26 (5.3)
Total	11506	8772 (76.2)	441 (5.0)	6694	4863 (72.6)	298 (6.1)

Notes.—(1) Figures from 1933-34 are for 'strangles seasons' (September to August); up to 1932-33 they are for financial years.

(2) The χ^2 test shows that there is a significant difference between the attack rates at Mona and Sargodha in all years from 1927, except 1927-28 and 1936-37. In the following years there is a significant difference in mortality rates: 1929-30, 1932-33 and 1938-39. The attack rate at Mona for 1927-40 is significantly higher than that from 1917-27.

(3) Figures in brackets are percentages.

Incidence of strangles in mules at Remount Depots by years

NOTES.—(1) Figures from 1933-34 are for 'strangles seasons'; up to 1932-33 they are for financial years.
(2) The χ^2 test shows that there is a significant difference between the attack rates at Mona and Sargodha in the following years: 1929-30, 1931-32, 1932-33, 1935-36, 1937-38 and 1939-40. There is no significant difference in the case mortality rates in mules.
(3) Figures in brackets are percentages.

TABLE III
Incidence of strangles in horses at Stud Farms by years

[illegible]

Problem of Equine Strangles in India

TABLE III—contd.

Incidence of strangles in horses at Stud Farms by years

Year	Probynabad			Coleyana			Ronala			Ahmednagar		
	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)	(1)	(2)	(3)
1930 .	240	28 (11.7)	8 (28.6)	150	75 (50.0)	1 (1.3)
1931 .	241	51 (21.2)	5 (9.8)	148	8 (5.4)	1 (12.5)	150	21 (14.0)	0 ..
1932 .	242	61 (25.2)	2 (3.3)	115	37 (32.2)	4 (10.8)	130	50 (38.5)	6 (12.0)
1933 .	246	69 (28.0)	1 (1.5)	126	15 (11.9)	0	130	22 (16.9)	1 (4.6)
1934 .	246	12 (4.9)	0 ..	137	55 (40.1)	8 (14.5)	122	52 (42.6)	5 (9.6)
1935	146	108 (74.0)	7 (6.5)	181	77 (42.6)	3 (3.9)	103	29 (28.2)	1 (3.5)
1936	147	20 (13.6)	3 (15.0)	221	21 (9.5)	1 (4.8)	96	8 (3.1)	0 ..
1937	148	79 (53.4)	1 (1.3)	202	26 (12.9)	0 ..	103	3 (2.9)	0 ..
1938	163	13 (8.0)	0 ..	220	239*	23
1939	140	2 (1.4)	0 ..	202	95 (47.0)	4 (4.2)
1940	147	6 (4.1)	0 ..	220	107 (48.6)	10 (9.4)
1941	147	3 (2.1)	0 ..	155	78 (50.0)	3 (3.9)
1942	158	6 (3.8)	0 ..	189	43 (22.8)	0
Total .	4436	830 (18.7)	47 (5.7)	1872	427 (22.8)	25 (5.9)	1590	686	44	834	180 (21.6)	13 (7.2)

Total of (2)=2123

Total of (3)=129 (6.1)

NOTES.—(1) Approximate young stock population present during the year.

(2) Case incidence of strangles.

(3) Case mortality.

*Figures in brackets represent percentages.

*Figure 239 includes young and adult cases, whereas 220 is the number of young stock alone.

TABLE IV

Young horse stock population by age groups

Year	Probynabad						Coleyana			
	Popu- la- tion	Age in years					Popu- la- tion	Age in years		
		One or less	To two	To three	To four	To five		One	Two	Three
1914 .	250	71	54	47	58	20
1915 .	230	62	70	53	42	3
1916 .	223	46	64	65	48	0
1917 .	241	76	45	61	58	1
1918 .	209	37	63	41	50	13
1919 .	202	47	31	60	35	26
1920 .	169	35	42	29	52	11
1921 .	201	56	31	42	28	44
1922 .	166	44	55	30	37	0
1923 .	175	39	44	52	30	10
1924 .	176	53	36	41	40	6
1925 .	182	44	51	33	39	15
1926 .	177	57	43	49	21	7
1927 .	182	56	54	41	30	1
1928 .	211	66	56	50	36	3	133	34	32	17
1929 .	227	69	65	54	38	1	133	79	24	30
1930 .	240	66	61	64	47	2	159	79	57	23
1931 .	241	76	53	54	49	4	140	73	33	31
1932 .	242	80	55	58	47	2	115	64	30	21
1933 .	246	74	66	56	47	3	125	73	38	14
1934 .	246	64	71	66	40	5	189	73	26	31
1935	137	76	33	28
1936	122	59	33	30
1937	144	82	33	29
1938	130	61	39	27
1939	150	89	29	32
1940	120	65	23	32
Total .	4436	1218 (27.5)	1120 (25.3)	1046 (23.6)	875 (19.7)	177 (4.0)	1738	963 (55.4)	430 (24.7)	345 (19.9)

Figures in brackets are percentages of the total.

SOME DIGESTIBILITY TRIALS ON INDIAN FEEDING STUFFS

XII. OILSEEDS, CAKES AND SOME FOOD GRAINS

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This paper records the determination of fundamental feeding data of oilseeds, oilseed cakes and some other food grains on heifers, conducted at Lyallpur under a scheme financed by the Imperial Council of Agricultural Research for three years, and the authors are indebted to the Council for the means afforded for carrying it out.

Six Montgomery heifers, the details concerning the ages and weights of which are shown in Table II, were employed as experimental animals throughout. Some difficulty was experienced at the beginning in designing the trials in such a way that the results could be submitted to statistical interpretation according to the modern methods. It was obviously impossible to conform strictly to such methods, and ultimately, in consultation with the Statistician to the Imperial Council of Agricultural Research, a plan was drawn up which, it was agreed, was most suitable for the feeding trials consistent with the number of animals.

In earlier trials [Lander, 1935 and 1936] when six animals were employed, it was customary to put all the animals on the same ration at one time. In the trials now conducted it was decided that for each separate ration the animals should be divided into two groups, designated A, B, F and C, D, E, and the rations to be investigated were serially numbered from 1 to 24 as shown in Table I. Each group of three animals was accordingly employed for each experimental period of 1½ months on one of these rations. This ensured that any particular ration would be fed to different groups of animals at different times of the year, thus eliminating to some degree seasonal variations and those in the animals treatment of the rations. The general outline of the whole plan of work based on this treatment is shown in Table I.

The general technique followed in these feeding trials has been recorded [Lander, 1928 and 1929] fully in the bulletins and publications on nutrition from the Agricultural Research Institute, Lyallpur, in connection with the work which has been carried on here during the last 20 years. It is not proposed, therefore, to redescribe these in any detail. A careful record was kept of the various amounts of rations fed daily to each of the animals and also of the dung and urine excreted.

Before the actual experimental trials with these concentrates were started, the six animals

were fed for one month on oat hay alone as a basal ration, oat hay being a maintenance ration. The animals were then given their respective rations of the concentrates, together with oat hay *ad lib* for a fortnight, following which the actual experimental trial for each feed was conducted for one month. Again a preparatory period of 14 days elapsed before the next experimental period of one month commenced, and so on throughout the period of three years.

During both the preparatory and experimental periods, all the animals were fed oat hay *ad lib* and were always given daily exercise under strict control.

During the course of the trials under report, the body weights of all the animals were taken daily and are recorded in Table II. These merit no particular comment, as the animals maintained and slightly increased their weights and also remained in good condition through the trials.

FEEDS EMPLOYED

Table III shows the chemical analyses of the feeds employed during the period under report.

Oilseed cakes

Sarson cake (*Brassica campestris* var. *sarson*). This cake is a common feed in the central parts of the Punjab and is much relished by cattle. Two pounds of cake (two pounds of the cake were fed in all cases except linseed and groundnut cake), after being soaked in water for two hours, were fed daily and eaten with relish. The digestibility data for this cake fed at two different times of the year on the two different sets of heifers correspond fairly well (Table IV).

Toria cake (*Brassica napus* var. *dichotoma*). *Toria* cake is commonly fed in the canal colonies of the Punjab. It has a slightly bitter taste and is somewhat richer in protein than *sarson* cake, and no trouble whatsoever was experienced with the animals fed on it. It is poorer in digestible fat than *sarson* cake and consequently shows a lower total content of digestible nutrients.

Taramira cake (*Eruca sativa*). This is a popular feed in the north-west Punjab and is supposed rightly or wrongly, to have a cooling effect. It is, however, distinctly pungent, has a very disagreeable odour, and the animals at first showed a disinclination to eat it, but they became accustomed to it after some time. In chemical composition it resembles *sarson* and *toria* cakes.

and the digestibility figures show that the total carbohydrates and dry matter in *taramira* are more digestible than those of *sarson* and *toria*, while the digestibility figures of the protein are much the same.

Linseed cake (*Linum usitatissimum*). This is not generally favoured by Punjab zemindars, but is extensively used in dairies and to some extent in the north-east districts of the Punjab.

Groundnut cake (*Arachis hypogaea*). Groundnut cake has only recently been introduced as a feeding stuff in the Punjab, but it is not yet extensively used, nor has it gained from stock owners the recognition it appears to merit. It is an excellent cake as regards taste and appearance, and of all the cakes so far tested it is richest in digestible proteins.

Oilseeds

Generally speaking, animals do not like oilseeds and perhaps this is why they are very rarely fed to cattle. *Taramira* (*Eruca sativa*) seed, which is distinctly pungent and has a very disagreeable odour, was not liked by the animals, and they fell off in condition; hence no reliable digestibility data could be obtained with this oilseed.

Linseed (*Linum usitatissimum*) also was not relished by the animals, and the consumption of the basal ration, oat hay, was much lower in this period than with other concentrates. Probably its mucilaginous character adversely affected the appetite.

Although linseed and *sarson* show identical figures for digestible fat and total digestible nutrients, yet on account of its mucilaginous character linseed is not comparable with *sarson* and is rightly not used as a concentrate feed but as a curative of digestive disorders.

Soybean (*Glycine hispida*), which stands in a category by itself, has a very high digestible protein content and a high digestible fat content. It is relished by animals and does not cause any digestive disturbances.

Grains, etc.

Guara (*Cyamopsis psoralioides*) is a leguminous *kharif* crop, and the grain is usually fed instead of gram, as it is cheaper.

Wheat *mamni* consists mostly of wheat screenings and small wheat grain usually infested with earcockle. It is very variable in character and composition, but because of its cheapness it finds favour with gavalas in towns.

Arhar (*Cajanus indicus*) is also a legume and compares very favourably with gram, but has not yet gained the recognition it appears to merit.

Moth (*Phaseolus aconitifolius*), *rawan* (*Dolichos lablab*) and *matri* (*Pisum sativum*) are also leguminous grains and are used in times of scarcity. All three are cheap sources of protein.

Oats (*Avena sativa*) is a concentrate of recognized importance.

Chari (*Andropogon sorghum*) and *bajra* (*Pennisetum typhoides*) are non-leguminous grains, common in the eastern and western Punjab.

DIGESTIBILITY DATA AND FOOD VALUES

The complete digestibility data obtained for the digestible constituents of the feed, the albuminoid ratio of the rations and the total digestible nutrients for all feeds are shown in Table IV. The figures are self-explanatory, and there is nothing particular to which attention needs to be drawn. A summary of the data presented is shown.

A point brought out in all digestibility trials is that the data obtained for ash, digestible fibre and nitrogen-free extract are likely to be misleading. As Maynard [1937] has pointed out some of the absorbed minerals are excreted through the gut, and there is no method of separating such minerals from the portion which originally failed to be absorbed. This being so, and as the re-excreted portion may be large as well as a variable part of the whole, it is obviously impossible to arrive at a figure of any value for the digestibility of most of the mineral elements. As Maynard records, data for digestible ash, which are frequently reported in connection with digestion trials, have no real significance. These facts have been noticed during many years' experience of digestibility trials at Lyallpur, and it has been decided accordingly not to present these figures.

Another important point which experience with feeding trials has emphasized is the difficulty of obtaining reliable figures for the digestibility of the fibre in feeds, such as oilseed cakes where the fibre content is as a rule low. The digestibility of the fibre in such cases is invariably masked by the fibre of the basal ration and abnormal figures are generally obtained. It is considered desirable, therefore, to group 'crude fibre' and 'nitrogen-free extract' together as 'total carbohydrates', a procedure which has also been followed by Watson and his colleagues [1939]. This procedure gives more satisfactory results and does not affect the figures for the total digestible nutrients or starch equivalents.

In the past it has been a common practice with research workers to work out the net energy values from the ascertained starch equivalents in a ration. Modern practice, however, is discarding this, and as Maynard points out, the values for total digestible nutrients must be considered the most practical measure, both for comparing feeds and as a basis for feeding standards. It must be recognized that the values for individual feeds are not constant and that they do not take account

of all losses during the passage of the feed through the alimentary canal, but these limitations can be lessened in practice by bearing in mind that digestibility is influenced by many factors and also by taking into account the losses to which digested nutrients are subject during the metabolic cycle. We have accordingly shown the total digestible nutrients in Table IV for all the feeds investigated.

In conclusion it may be pointed out that the data now presented represent fundamental data, the value of which can only be properly utilized if applied in the computing of rations on the basis of digestible nutrients which the various rations contain and of economic factors connected with the prices of feeding stuffs at various times of the year.

SUMMARY

Feeding values of oilseeds, oilseed cakes and some other food grains are summarized below:—

Name of the feed	Dry matter per 100 lb. of feed	Total digestible nutrients per 100 lb. of feed	Digestible protein per 100 lb. of feed	Nutritive ratio 1:
	lb.	lb.	lb.	
<i>Oilseed cakes</i>				
Sarson cake . . .	93.56	81.60	25.70	2.3
Toria cake . . .	96.21	73.95	30.47	1.5
Taramira cake . . .	89.80	85.55	23.92	2.2
Linseed cake . . .	94.39	82.57	23.58	2.0
Groundnut cake . . .	93.74	79.09	31.11	1.6
<i>Oilseeds</i>				
Linseed . . .	94.56	108.79	14.78	6.6
Sarson seed . . .	96.40	104.77	19.82	4.5
Soybean . . .	92.50	78.72	34.65	1.4
<i>Grains</i>				
Gram . . .	91.88	79.60	12.43	5.5
Maize . . .	91.86	70.51	5.37	12.3
Barley . . .	91.85	70.75	6.69	9.7
Wheat . . .	91.02	83.97	5.75	13.7
Wheat <i>manvi</i> . . .	94.15	54.88	0.52	7.6
Wheat bran . . .	89.08	70.79	8.80	7.1
Guara . . .	92.60	72.49	28.75	1.6
Bayra . . .	90.03	54.27	4.86	10.7
Arhar . . .	91.03	67.71	13.11	4.2
Oats . . .	90.54	66.90	4.44	14.1
Chari . . .	90.43	73.06	6.41	10.2
Moth . . .	91.35	72.56	17.40	3.2
Kanona . . .	89.84	62.30	18.57	2.4
Matri . . .	89.24	68.69	18.40	2.7

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TABLE I

Plan of the feeding experiments, six animals same age; breed and weight, (say A, B, C, D, E, F)

Treatments numbered 1 to 24

Concentrates	Period of experiments	Treatments	Animals	
			AEF	ODE
A. Oil-cakes	1½ months	Treatments . . .	1	2
		Do . . .	2	3
		Do . . .	3	1
		Do . . .	4	5
	Do	Do . . .	5	6
		Do . . .	6	4

Total 6 treatments; (oilcakes) time taken 9 months

B. Oil-seeds	1½ months	Treatments . . .	7	8
		Do . . .	8	9
		Do . . .	9	10
		Do . . .	10	7

Total 4 treatments; (oilseeds) time taken 6 months

C. Grains, etc	1½ months	Treatments . . .	11	12
		Do . . .	12	13
		Do . . .	13	11
		Do . . .	14	15
Oats	Do	Do . . .	15	16
		Do . . .	16	14
		Do . . .	17	18
		Do . . .	18	19
Wheat	Do	Do . . .	19	17
		Do . . .	20	21
		Do . . .	21	22
		Do . . .	22	20
Matri	Do	Do . . .	23	24
		Do . . .	24	23
		Do . . .	25	26
		Do . . .	26	25

Total 14 treatments; (grains) time taken 21 months

TABLE II

Particulars regarding the heifers

Heifer No.	Age at the beginning of the experiments (1 April 1939)		Average body weight in lb.			
			1 April 1939	1 Jan. 1940	1 Jan. 1941	14 March 1942
	Years	Months				
Group I						
A . . .	3	6	708	779	815	840
B . . .	3	6	658	763	816	854
F . . .	3	4	701	820	850	880
Group II						
C . . .	3	7	728	842	890	912
D . . .	3	4	580	728	760	810
E . . .	3	5	695	890	890	942

TABLE III
Chemical composition of the feeds as fed

Feed	Mois- ture per cent	Dry matter per cent	Ash per cent	Fat per cent	Crude fibre per cent	Protein per cent	Total Carbohy- drates per cent	Nitro- gen-free extract per cent	CaO per cent	P ₂ O ₅ per cent	Ratio CaO: P ₂ O ₅ 1:
1940											
Oat hay	6.40	93.60	6.65	2.62	34.94	4.35	..	45.04	0.2587	0.1016	1.35
Do.	6.77	93.23	8.45	1.33	36.76	5.25	..	41.44	0.2801	0.2076	1.35
Sarson cake	6.44	93.56	8.94	12.81	10.21	29.56	..	32.04	1.3014	1.1468	0.806
Do.	6.56	94.44	8.81	14.22	10.02	28.19	..	32.20	1.2898	2.3170	0.557
Toria cake	3.79	96.21	7.53	8.58	9.75	35.00	..	35.35	1.0248	2.0456	0.387
Tarariva cake	10.20	89.80	7.49	11.64	9.80	34.88	..	28.67	1.0458	2.0039	0.732
Do.	6.42	93.58	7.60	11.63	8.78	33.25	..	32.34	1.2723	2.4200	0.526
Linseed cake	5.61	94.39	9.34	6.17	9.38	27.88	..	42.12	0.7672	1.6364	0.469
Do.	5.61	94.39	9.44	4.20	9.05	28.94	..	42.76	0.6944	1.6191	0.429
Groundnut cake	6.26	93.74	5.39	6.06	15.24	37.57	..	29.48	0.2128	0.9937	0.214
1941											
Oat Hay	5.88	94.12	6.77	1.71	33.98	4.22	..	47.44	0.3360	0.2356	1.4260
Linseed	5.44	94.56	4.96	34.14	6.42	18.18	..	30.86	0.3360	1.3450	0.249
Taramira seed	5.36	94.64	7.72	32.11	6.27	26.69	..	21.85	0.6720	1.4140	0.47
Soybeans	7.50	92.50	5.65	16.10	5.59	38.50	..	26.66	0.5855	1.6408	0.320
Sarson seed	3.60	96.40	5.81	42.08	6.02	20.80	..	21.59	0.6658	1.5280	0.437
Gram	3.12	91.88	2.42	4.45	6.90	18.63	..	60.08	0.3975	0.9031	0.4400
Maize	8.15	91.85	1.70	3.03	2.62	9.70	..	75.41	0.0616	0.8397	0.0734
Barley	9.15	91.85	2.47	2.67	6.20	9.80	..	71.12	0.0700	0.3044	0.2299
Wheat	8.83	91.62	1.74	1.70	1.73	6.62	..	76.83	0.1848	0.7921	0.1631
Wheat mami	5.82	94.18	14.56	5.66	11.40	11.58	..	50.98	3.3040	0.9674	3.4140
Wheat bran	10.92	89.68	6.80	4.44	12.33	11.50	..	54.11	0.2310	1.9680	0.1173
1942											
Guara grain	7.40	92.60	..	4.36	..	38.38	45.61	..	0.630	1.842	0.47
Bayra grain	7.29	90.03	..	4.63	..	9.73	72.69	..	0.140	0.925	0.15
Arhar grain	8.37	91.63	..	1.75	..	18.75	64.29	..	0.280	0.782	0.36
Oat grain	9.45	90.54	..	6.04	..	8.14	71.03	..	0.398	0.807	0.38
Chari grain	9.57	90.43	..	2.25	..	13.75	71.90	..	0.112	0.765	0.15
Rauzan grain	10.16	89.84	..	1.42	..	23.98	58.53	..	0.260	0.998	0.26
Mati grain	10.76	89.24	..	0.85	..	23.56	57.76	..	0.714	0.618	1.16
Moth grain	8.65	91.35	..	0.76	..	22.60	63.77	..	0.320	0.771	0.41

TABLE IV
Digestibility data

Period	Concentrate eaten per day lb.	Oak hay eaten per day lb.	Digestibility coefficients				Digestible nutrients per 100 lb. of the feed				Abm. ratio	Total digestible nutrients	
			Holter No.	Digestibility coefficients			Digestible nutrients per 100 lb. of the feed						
				Dry matter	Fat	Protein	Total carbohydrates	Dry matter	Fat	Protein			Total carbohydrates
<i>Oat hay alone as basal ration</i>													
16-4-39 to 30-4-39	..	11-4	A	55-53	73-32	42-00	57-32	52-02	1-03	1-84	44-14	27-5	52-12
Ditto	..	10-5	B	53-22	67-86	37-28	55-58	49-81	1-61	1-63	44-95	30-3	50-45
Ditto	..	10-9	C	60-20	70-37	43-21	62-96	56-35	1-88	1-88	50-41	29-1	50-31
Ditto	..	10-5	D	53-82	65-50	33-78	56-63	50-37	1-74	1-47	39-51	33-7	50-72
Ditto	..	9-4	E	54-10	72-00	39-07	60-00	50-64	1-92	1-70	45-10	29-1	50-92
Ditto	..	12-0	F	56-63	74-20	40-32	58-00	53-00	1-92	1-75	47-25	29-5	52-12
<i>Sesoon cake</i>													
15-5-39 to 14-6-39	2	10-0	A	75-82	89-86	80-60	69-81	70-85	12-78	25-26	39-47	2-3	81-35
Ditto	2	9-0	B	75-88	91-56	92-34	67-79	70-92	13-02	26-03	38-62	2-3	81-78
Ditto	2	10-0	C	76-38	89-02	91-52	68-59	71-38	12-66	25-80	38-96	2-3	81-06
16-8-39 to 15-9-39	2	10-4	D	75-12	91-48	89-73	66-54	70-21	13-01	25-31	38-09	2-3	81-50
Ditto	2	9-3	E	75-01	91-42	91-73	65-33	70-11	12-00	25-88	37-58	2-3	81-48
Ditto	2	10-9	F	77-12	93-24	91-88	65-71	72-08	13-26	25-91	39-01	2-3	82-52
<i>Toria cake</i>													
15-5-39 to 14-6-39	2	10-7	C	73-10	90-32	86-92	62-87	70-33	7-75	30-44	38-36	1-5	74-02
Ditto	2	8-9	D	72-54	88-12	84-72	64-79	69-78	7-39	29-65	39-23	1-6	73-35
Ditto	2	10-4	E	71-75	87-99	83-10	64-07	69-02	7-55	29-08	38-90	1-6	72-85
4-7-39 to 31-7-39	2	8-4	F	72-11	91-72	85-23	61-78	69-38	7-87	29-83	37-87	1-5	73-22
Ditto	2	8-4	A	74-00	86-34	88-40	63-54	74-19	8-18	30-94	38-66	1-5	75-74
Ditto	2	9-9	B	73-70	90-54	86-68	64-08	70-91	7-77	30-34	38-88	1-5	72-50
<i>Taramira cake</i>													
4-7-39 to 31-7-39	2	10-9	C	81-64	76-89	90-53	81-66	76-41	8-95	30-12	34-39	1-8	62-35
Ditto	2	8-1	D	80-88	80-15	86-22	88-06	81-32	9-32	33-67	40-21	2-2	73-76
Ditto	2	11-6	E	85-16	84-36	84-24	94-43	79-71	9-81	28-01	38-87	2-2	86-74
16-8-39 to 15-9-39	2	8-3	A	82-30	88-24	90-31	81-09	77-02	10-26	30-03	32-32	1-9	84-15
Ditto	2	8-3	B	84-82	89-75	87-84	88-64	79-36	9-97	29-14	30-43	2-0	89-76
Ditto	2	9-8	F	84-18	82-84	82-80	82-92	78-77	9-71	27-62	35-20	2-2	85-22
<i>Linsed cake</i>													
1-10-39 to 31-10-39	2	9-2	A	86-48	82-35	83-50	96-20	81-64	5-08	24-24	49-55	3-5	83-51
Ditto	2	9-9	B	86-44	78-88	88-01	96-90	80-70	4-87	24-10	49-39	2-5	89-74
Ditto	2	10-0	F	87-63	82-30	85-30	..	82-72	5-08	23-36	..	2-7	..

TABLE IV—contd.

Period	Concentrate eaten per day lb.	Oat hay eaten per day lb.	Heifer No.	Digestibility coefficients				Digestible nutrients per 100 lb. of the feed				Albuminoid ratio 1:	Total digestible nutrients		
				Lined cake—contd.			Total carbohydrates	Lined seed			Dry matter			Protein	Total carbohydrates
				Dry matter	Fat	Protein		Dry matter	Fat	Protein					
3-1-40 to 31-1-40	3	11-1	C	86-76	80-32	85-62	97-43	81-88	4-97	23-72	50-18	2-6	82-41		
Ditto	3	9-8	D	82-51	78-81	82-30	92-19	77-90	4-86	22-44	47-47	2-6	79-27		
Ditto	3	9-8	E	86-58	76-22	85-43	96-93	80-93	4-70	23-39	42-92	2-6	82-25		
Groundnut cake															
1-10-39 to 31-10-39	3	12-0	C	82-90	76-48	90-01	85-34	77-71	4-64	32-20	38-17	1-5	78-05		
Ditto	3	10-0	D	81-67	82-50	84-80	86-13	76-55	5-00	30-03	38-52	1-7	77-75		
Ditto	3	11-7	E	80-82	80-90	82-65	81-76	75-76	4-90	29-57	31-80	1-7	77-58		
16-11-39 to 15-12-39	3	7-9	A	84-43	95-00	80-01	87-43	79-14	5-76	31-86	31-10	1-7	81-72		
Ditto	3	8-0	B	80-73	90-89	83-99	81-88	76-67	5-81	31-84	38-62	1-6	79-33		
Ditto	3	10-0	F	83-63	92-30	87-06	85-24	78-33	5-59	31-15	38-12	1-6	79-70		
Lined seed															
16-2-40 to 15-3-40	1-0	5-6	A	77-13	90-68	83-64	67-48	72-93	30-95	15-21	25-16	6-3	107-55		
Ditto	1-0	6-0	B	77-39	90-94	71-66	70-00	73-17	31-04	18-03	20-10	7-5	106-64		
Ditto	1-0	4-8	F	79-24	98-10	88-60	62-59	74-93	33-50	16-11	23-34	6-0	112-18		
Soybeans															
1-5-40 to 31-5-40	2-0	10-0	A	73-51	76-40	90-38	58-91	68-00	12-30	34-80	19-00	1-3	78-77		
Ditto	2-0	10-0	B	72-16	79-19	89-74	57-21	66-75	12-75	34-55	18-45	1-4	78-97		
Ditto	2-0	10-0	F	70-95	76-71	89-87	58-00	65-64	12-35	34-60	18-72	1-4	78-41		
Sargol seed															
1-5-40 to 31-5-40	1-0	10-0	C	87-66	69-72	92-52	79-74	84-50	29-30	19-25	22-10	4-7	104-86		
Ditto	1-0	8-8	D	74-82	65-90	96-64	86-24	72-12	27-73	20-10	23-90	4-4	103-80		
Ditto	1-0	10-0	E	80-28	70-22	95-64	78-48	77-39	29-55	20-10	21-75	4-5	105-65		
Grams															
26-6-40 to 14-7-40	3-0	7-7	A	81-45	78-04	71-90	85-58	74-86	3-47	12-97	57-32	5-0	77-14		
Ditto	3-0	8-0	B	81-58	85-46	65-48	86-25	74-96	3-80	11-80	57-76	5-6	77-21		
Ditto	3-0	8-0	F	88-71	80-30	70-28	84-49	81-51	3-57	12-68	63-28	5-6	83-05		
16-9-40 to 15-10-40	4-0	10-0	C	82-10	89-35	67-60	86-70	75-43	3-93	12-19	58-00	5-6	78-17		
Ditto	4-0	8-0	D	88-44	88-76	72-16	93-78	81-36	3-95	13-01	62-81	5-6	83-73		
Ditto	4-0	9-0	E	82-26	85-06	66-06	87-75	75-60	3-79	11-91	58-77	5-7	78-30		
Moate															
24-6-40 to 14-7-40	2-0	9-0	C	75-22	86-68	56-48	77-73	60-10	2-63	5-48	60-19	12-1	71-13		
Ditto	2-0	6-0	D	73-14	79-86	57-73	75-62	67-20	2-42	5-60	68-56	11-5	69-14		
Ditto	2-0	6-0	E	74-84	75-65	50-00	78-40	68-75	2-29	4-85	60-70	13-6	70-30		
1-8-40 to 31-8-40	3-0	7-4	A	75-22	85-73	57-73	77-48	60-10	2-60	5-60	60-00	11-8	70-98		
Ditto	3-0	7-6	D	60-45	82-43	55-50	70-71	63-81	2-50	5-38	54-75	12-4	71-47		
Ditto	3-0	7-5	F	75-62	82-22	54-60	76-97	67-64	2-49	5-30	59-60	12-3	70-06		

Barley

[illegible]

TABLE IV—contd.

Period.	Concentrate eaten per day lb.	Oat hay eaten per day lb.	Hofier No.	Digestibility coefficients				Digestible nutrients per 100 lb. of the feed				Albuminoid ratio	Total digestible nutrients
				Digestibility coefficients			Total carbohydrates	Dry matter	Fat	Protein	Total carbohydrates		
				Dry matter	Fat	Protein							
Oats													
1-9-41 to 30-9-41	•	3	A	68.11	77.31	50.25	70.22	81.67	4.67	4.09	40.88	14.8	64.48
Ditto	•	3	B	69.22	77.31	49.14	70.86	82.67	4.67	4.00	50.33	15.2	64.84
Ditto	•	3	F	71.05	71.69	53.19	74.02	84.33	4.33	4.23	54.00	14.7	68.07
1-12-41 to 23-12-41	•	3	C	68.84	80.01	57.94	71.19	86.08	3.91	5.60	51.33	12.0	65.13
Ditto	•	3	D	72.38	81.97	52.96	78.13	86.33	4.00	4.00	56.33	14.3	69.90
Ditto	•	3	E	71.89	81.97	54.11	76.74	85.87	4.00	4.67	55.33	13.8	69.00
Charl													
1-9-41 to 30-9-41	•	3	C	76.67	61.34	48.52	85.98	89.33	1.38	6.67	61.82	9.3	71.60
Ditto	•	3	D	76.30	66.67	46.55	46.41	69.00	1.50	6.40	62.13	10.2	71.91
Ditto	•	3	E	80.36	69.34	46.04	90.86	72.67	1.56	6.33	65.33	10.9	75.17
27-10-41 to 14-11-41	•	3	A	78.50	59.48	49.63	88.75	70.67	2.04	6.47	62.38	10.3	73.39
Ditto	•	3	B	75.96	68.23	47.63	85.93	68.33	2.34	6.21	60.33	10.6	71.81
Ditto	•	3	F	73.74	65.00	48.86	94.93	66.33	2.25	6.37	66.67	9.7	78.10
Moth													
27-10-41 to 14-11-41	•	Sick	C	85.75	73.69	78.18	90.97	78.33	0.56	17.67	58.01	3.4	76.94
Ditto	•	3	D	83.93	77.63	75.22	88.32	76.67	0.59	17.00	56.33	3.4	74.66
Ditto	•	3	E	81.38	78.00	78.95	81.28	72.33	0.78	17.67	48.67	2.9	68.10
1-12-41 to 23-12-41	•	3	A	84.01	79.00	78.95	89.63	74.67	0.72	17.67	53.67	3.1	72.96
Ditto	•	3	F	78.76	74.00	75.96	83.50	70.00	0.74	17.00	49.99	3.0	68.66
Kawan													
8-1-42 to 31-1-42	•	3	A	71.24	62.68	78.02	71.14	64.00	0.89	18.67	41.64	2.3	62.31
Ditto	•	3	B	69.35	58.45	78.02	69.49	62.33	0.83	18.67	40.67	2.3	61.21
Ditto	•	3	F	68.64	58.45	74.72	71.76	61.67	0.83	17.88	42.00	2.5	61.75
15-2-42 to 14-3-42	•	3	C	70.12	62.68	75.22	75.17	63.00	0.89	18.00	44.00	2.6	64.00
Ditto	•	3	D	69.76	59.34	78.02	76.32	62.67	0.80	18.67	44.67	2.5	65.14
Ditto	•	3	E	67.53	60.56	76.59	68.90	60.67	0.86	18.35	40.33	2.3	60.60
Matni													
8-1-42 to 31-1-42	•	Sick	C	76.95	80.00	77.80	82.51	89.67	0.68	18.33	47.66	2.7	67.52
Ditto	•	3	D	81.05	78.53	75.60	88.45	72.33	0.67	17.67	51.09	2.9	69.27
Ditto	•	3	E	78.29	78.82	80.64	80.97	70.88	0.67	19.00	46.77	2.5	67.98
15-2-42 to 14-5-42	•	3	B	78.37	78.83	77.80	82.65	68.67	0.67	18.33	49.01	2.8	68.85
Ditto	•	3	F	82.07	77.63	79.24	87.24	73.33	0.66	18.67	50.39	2.8	70.55

SIMPLIFICATION OF THE GERBER TEST USED FOR TESTING FAT IN MILK

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(Received for publication on 8 June 1943)

In India the Gerber method for testing fat in milk is almost universally used. The method embodies accuracy with simplicity. Its only drawback is that it cannot always be carried out under village conditions, without an expensive outfit. The war conditions have added to the difficulty of obtaining the equipment at present. According to the standard method, centrifuging is essential. Purchase of such a costly machine is usually beyond the means of small producers, especially so at present. The following preliminary studies were, therefore, carried out with a view to modifying the technique of the Gerber test so that by using the same standard glassware, the costly centrifuge might be dispensed with, without reducing the accuracy of the results.

EXPERIMENTAL

(i) The effect of keeping test solution for different time intervals on the Gerber test

This part of the experiment was planned with the object of determining how long a fieldman could safely keep the mixture of milk, acid and alcohol in a fit condition before taking the reading, and without in any way impairing the accuracy of the test, in case a centrifuge was not readily available. Reaction mixtures containing 10 ml. of sulphuric acid (sp. gr. 1.82), 11 ml. of milk and 1 ml. of amyl alcohol were prepared and kept at room temperature for different intervals of time as shown in Table I. Readings were taken after each time interval, after placing butyrometers in a water-bath at 160°F., both with and without centrifuging. The results for cow and buffalo milk are shown in Table I.

These results show that when the conventional method of making the Gerber test is followed and the tubes dipped in a water bath at 160°F. for five minutes before centrifuging, it is immaterial after what interval between the mixing and dipping of the reaction mixture the test is read. It is also interesting to note that, by merely keeping the butyrometers containing reaction mixture at room temperatures for about eight hours and then dipping them in a water bath at 160°F., correct readings can be obtained. At shorter intervals than eight hours, the results were not consistent.

(ii) Effect of adding amyl alcohol at a later stage

In the standard method the alcohol is added after the milk and just before the mixture is shaken

TABLE I
Effect of time interval on the Gerber test

Sample No.	Time interval in hours	Cow whole milk		Buffalo whole milk	
		Reading after keeping in water bath at 160°F.		Reading after keeping in water bath at 160°F.	
		5 min.	5 min and then centrifuging	5 min.	5 min and then centrifuging
		(Initial fat test 4.3 per cent)		(Initial fat test 6.2 per cent)	
1	2	3.8	4.3	6.0	6.2
2	4	4.0	4.3	5.9	6.2
3	6	3.9	4.3	6.1	6.2
4	8	4.3	4.3	6.2	6.2
5	24	4.3	4.3	6.2	6.2
6	48	4.3	4.3	6.2	6.2
7	72	4.3	4.3	6.3	6.3
8	96	4.3	4.3	6.3	6.3
9	120	4.4	4.4	6.2	6.2
10	144	4.3	4.3	6.2	6.2
11	168	4.3	4.3	6.2	6.2
12	192	4.3	4.3	6.2	6.2
13	216	4.3	4.3	6.2	6.2
14	240	4.3	4.3	6.2	6.2

vigorously. Trials were carried out to see what the effect was of adding amyl alcohol after allowing the mixture of milk and acid to stand over different periods and just before putting the butyrometers in the bath. In this case also the readings were taken after each time interval, after placing in a water-bath both with and without centrifuging. The results are given in Table II.

The results showed that if sufficient time is not allowed between adding of the amyl alcohol and putting the sample in the bath the fatty layer does not separate quick enough to give correct reading and centrifuging is, therefore, necessary.

(iii) Effect of temperature on the Gerber test

For taking readings in the standard Gerber test the temperature of the reaction mixture recommended is 140°-160°F. To test the effect of temperature on the Gerber test, three temperatures were tried out, viz. 120°F., 149°F. and 160°F.

TABLE II

Effect of adding amyl alcohol at a later stage

Sample No.	Time interval in hours	Cow whole milk		Buffalo whole milk	
		Reading after keeping in water bath at 160°F.		Reading after keeping in water bath at 150°F.	
		5 min.	5 min. and centrifuging.	5 min.	5 min. and centrifuging.
Series I.		(Gerber test 4.4 per cent fat)			
1	2	2.8	4.4		
2	4	3.4	4.4		
3	6	3.0	4.3		
4	8	3.4	4.3		
5	26	3.9	4.4		
6	28	3.9	4.3		
7	30	4.0	4.3		
Series II.		(Gerber test 4.3 per cent fat)		(Gerber test 6.6 per cent fat)	
8	24	3.0	4.3	4.6	6.6
9	48	3.2	4.3	6.0	6.6
10	72	3.4	4.3	6.2	6.6
11	98	3.5	4.3	6.0	6.6
12	120	3.8	4.3	6.4	6.6
13	144	3.5	4.3	6.0	6.6
14	168	3.6	4.3	6.4	6.6

In each case a series of mixtures of milk, sulphuric acid and amyl alcohol were prepared in the normal way, in triplicate, for each time interval indicated below. The triplicate tubes were examined as follows: the first tube was kept at the particular temperature to be tried for five minutes before testing; the second tube was centrifuged and fat read; while the third tube was centrifuged, immersed in a water bath for five minutes at the temperature under observation and the fat percentage then read. The results are shown in Table III.

It will be seen that merely keeping the sample in the water bath at 120°F. gave rather a low reading. If, however, the sample is kept in the bath at this temperature after centrifuging, no change in the reading is noted. The same tendency was noted when employing 149°F. All the fat layer did not separate out by merely immersing the sample in the bath but it was necessary to centrifuge it. When using 160°F. the reading tended to equalize, after about eight hours, with the reading obtained by the standard method. Hence it could be concluded that if the ordinary Gerber test is carried out after allowing the reaction mixture to stand for eight hours at room temperature and then immersing it in the bath at 160°F. for five minutes the reading obtained would be the same as that obtained by centrifuging it immediately after mixing the solutions.

TABLE III

Effect of using different temperatures in Gerber test

Sample No.	Time interval in hours	Temperature 120°F.			Temperature 149°F.			Temperature 160°F.		
		Keeping in water-bath alone.	Centrifuging alone	Centrifuging and keeping in bath	Keeping in water-bath alone	Centrifuging alone	Centrifuging and keeping in bath	Keeping in water-bath alone	Centrifuging alone	Centrifuging and keeping in bath
Cow whole milk (Gerber test 4.2 per cent fat)										
1	0	..	4.1	4.1	..	4.1	4.1	..	4.1	4.2
2	2	3.7	4.1	4.1	3.8	4.1	4.2
3	4	3.9	4.1	4.1	3.8	4.1	4.1	3.9	4.1	4.2
4	6	3.9	4.1	4.1	3.9	4.1	4.1	3.9	4.1	4.2
5	24	3.9	4.1	4.1	3.9	4.1	4.1	4.1	4.1	4.2
6	26	3.9	4.1	4.1	3.9	4.1	4.1	4.1	4.1	4.2
Buffalo milk (Gerber test 6.4 per cent fat)										
1	0	..	6.3	6.3	..	6.3	6.3	..	6.3	6.4
2	4	6.0	6.3	6.3	6.0	6.3	6.3	6.1	6.3	6.4
3	8	6.0	6.3	6.3	6.2	6.3	6.3	6.3	6.3	6.4
4	24	6.2	6.3	6.3	6.2	6.3	6.3	6.3	6.3	6.4
5	28	6.2	6.3	6.3	6.2	6.3	6.3	6.3	6.3	6.4
6	32	6.2	6.3	6.3	6.2	6.3	6.3	6.3	6.3	6.4

(iv) *Effect of varying the period of heating at different temperatures on the Gerber test*

In practice probably waiting for eight hours before taking the reading may be a disadvantage. Trials were carried out to see if this interval could be considerably reduced by either increasing the period of heating or by still further raising the temperature.

For these trials, mixtures of milk acid and amyl alcohol were prepared as usual and kept at

room temperature. Before testing they were immersed in the water-bath at 120°, 149°, 160°, 165°, 175°, 180°, 185°, 190° and 195°F., for different intervals as indicated in Table IV.

The results showed that the temperatures of 120°F. and 149°F. were too low to be employed for these experiments. As the temperature was raised to 160°F. and upwards, correct readings were obtained at varying intervals of time. The higher the temperature the shorter was the time after which the reading could be taken.

TABLE IV

Effect of varying periods of heating at different temperatures

Sample No.	Time interval in hours	Cow whole milk			Buffalo whole mil		
		Time of immersing in water-bath			Time of immersing in water-bath		
		10 min.	15 min.	20 min.	10 min.	15 min.	20 min.
A. 120°F.							
		(Gerber test 4.1 per cent fat)					
1	0	3.6	3.8	3.9			
2	2	3.9	3.9	4.0			
3	4	3.9	4.0	4.0			
4	6	3.9	4.0	4.0			
5	8	3.9	4.0	4.0			
6	24	3.9	4.0	4.0			
B. 149°F.							
		(Gerber test 4.1 per cent fat)					
1	1	3.6	3.9	4.0			
2	3	4.0	4.0	4.0			
3	5	4.0	4.0	4.0			
four	7	4.0	4.0	4.0			
5	24	4.0	4.0	4.0			
C. 160°F.							
		(Gerber test 4.6 per cent fat)					
1	1	4.4	4.4	4.1			
2	3	4.5	4.5	4.6			
3	5	4.6	4.6	4.6			
4	7	4.6	4.6	4.6			
5	24	4.6	4.6	4.6			
D. 165°F.							
		(Gerber test 3.6 per cent fat)					
1	1	3.3	3.4	3.4			
2	1½	3.5	3.5	3.5			
3	2	3.5	3.5	3.6			
4	2½	3.5	3.6	3.6			
5	3	3.5	3.6	3.6			
6	3½	3.5	3.6	3.6			
7	4	3.6	3.6	3.6			
8	4½	3.6	3.6	3.6			
E. 175°F.							
		(Gerber test 4.5 per cent fat)					
1	1	4.3	4.4	4.4			
2	1½	4.4	4.4	4.4			
3	2	4.4	4.5	4.5			
4	2½	4.5	4.5	4.5			
5	3	4.5	4.5	4.5			
6	3½	4.5	4.5	4.5			
7	4	4.5	4.5	4.5			

Sample No.	Time interval in hours	Cow whole milk			Buffalo whole milk		
		Time of immersing in water-bath			Time of immersing in water-bath		
		10 min.	15 min.	20 min.	10 min.	15 min.	20 min.
F. 180°F.		(Gerber test 4.7 per cent fat)			(Gerber test 6.4 per cent fat)		
1	1	4.6	4.6	4.6	6.3	6.3	6.3
2	1½	4.6	4.6	4.7	6.3	6.3	6.3
3	2	4.7	4.7	4.7	6.4	6.4	6.4
4	2½	4.7	4.7	4.7	6.4	6.4	6.4
G. 185°F.		(Gerber test 4.6 per cent fat)			(Gerber test 6.4 per cent fat)		
1	1	4.4	4.5	4.5	6.3	6.3	6.3
2	1½	4.5	4.5	4.6	6.3	6.4	6.4
3	2	4.6	4.6	4.6	6.4	6.4	6.4
4	2½	4.6	4.6	4.6			
5	3	4.6	4.6	4.6			
6	3½	4.6	4.6	4.6			
7	4	4.6	4.6	4.6			
H. 190°F.					(Gerber test 6.8 per cent fat)		
1	1				6.7	6.7	6.8
2	1½				6.8	6.8	6.8
3	2				6.8		
I. 195°F.		(Gerber test 4.7 per cent fat)			(Gerber test 6.8 per cent fat)		
1	½				6.6	6.7	6.7
2	1	4.6	4.6	4.7	6.7	6.8	6.8
3	1½	4.7	4.7	4.7	6.8	6.8	6.8
4	2	4.7	4.7	4.7			60°F 8

At 160°F. the correct reading corresponding to the control Gerber test was obtained after three hours by keeping the butyrometer for 20 minutes in the bath; at 180°F. the interval was reduced to two hours; at 190°F., to 1½ hours and at 195°F., to one hour. Hence any of these temperatures could be selected, but temperatures between 175°F. to 190°F. are likely to prove more convenient on account of saving in time. It should be noted that it is essential to completely dip the butyrometers in water bath in all these cases.

CONCLUSIONS

(i) It is not necessary to centrifuge a sample immediately it is prepared when estimating fat by the Gerber method, provided the precaution is observed that such a mixture is dipped for five minutes in water-bath at 160°F. before taking the

reading. Under these circumstances even after 10 days the correct reading can be obtained.

(ii) If the butyrometers are allowed to stand at room temperature, in about eight hours or above, the fat layer gets enough time to separate out from the remainder of the mixture and it is possible to dispense with centrifuging entirely.

(iii) It is possible to shorten the time before correct reading can be obtained, and at the same time dispense with the centrifuge, by raising the temperature of the bath and prolonging the time of dipping. Thus, for example, it is found that at 190°F. reading could be obtained after an interval of only one hour.

(iv) These modifications in the standard Gerber method makes possible its regular use by small producers.

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(Received for publication on 26 May 1943)

ON receiving a report from the Veterinary Investigation Officer, North-West Frontier Province, in 1939 that several animals in a herd had been poisoned by plants on which they had grazed in a particular area near Parachinar, feeding trials with the suspected plants were undertaken on hill bulls, sheep and goats.

This article presents observations on symptom and *post mortem* changes after *Nerium odorum* had been fed to the animals.

Nerium odorum (Punjab—*kaner*: Pungal—*karachi*: Baluchistan—*gandeli*) is a large, glabrous, evergreen shrub with a milky juice, bearing sweet-scented rose-red or white flowers. According to Kirtikar and Basu [1938] it is extensively cultivated throughout the greater part of India as an ornamental plant in the garden. In the wild state it is found in the upper Gangetic plains, on the Himalayan ranges from Nepal to Kashmir up to an elevation of 6,500 ft., on the salt ranges, and in Waziristan, Baluchistan, Central and South India.

SYMPTOMS OF POISONING

Hill bull. One hill bull was drenched with a water suspension of 200 gm. of leaves, stem and flowers of the plant. After a short time, the animal began to grunt and retch. Micturition and defecation became very frequent. (In 30 minutes there were four micturitions and three defecations). After about ten minutes, the pulse became weak and the respiration somewhat rapid. Death occurred in convulsions in about 35-40 minutes after drenching.

Goat. A preliminary feeding of 10 gm. of the plant caused trembling throughout the entire body. After two hours, the trembling lessened, but continued for another two hours. When similar dose was given on the following day, the animal's temperature became sub-normal, and death occurred in about two hours.

Sheep. In sheep the only symptom, after 10 gm. of the plant, was laboured respiration. Death occurred during the night.

POSTMORTEM CHANGES

In all cases there was a slight congestion of the fundus portion of the abomasum and duodenum, and the contents of the rectum were semi-fluid. The abdominal cavity in sheep contained 500 c.c. of faintly blood-tinged fluid.

EFFECT OF POISONING ON MEAT

The heart and muscles from various parts of the body of both sheep and goats that died of *kaner* poisoning were fed separately to two dogs for two days without any ill effects. The heart (52 gm.) and muscle (49 gm.) were fed to dogs weighing 52 and 48 lb. respectively.

TOXICITY OF THE PLANT

The plant appears to be highly toxic. Snipe [1887] fed two ounces of the leaves night and morning for three days to a horse and observed symptoms of dull abdominal pain, anorexia and yellowness.

Kobert [1902] found that meat roasted on skewers of *kaner* wood had been responsible for death in soldiers. According to Walsh [1909], 'a single growing top of the plant proved fatal' to horses and cattle.

From the table given below it will be seen that the minimum lethal dose is 4 gm. per kg. body weight for bulls; 0.3 gm. for sheep and 1.2 gm. for goats. Steyn [1929] found that the minimum lethal dose of the dried leaves and flowers was 5 gm. per kg. body weight for the rabbit. It is stated that a few leaves may cause death in human beings [Von Itallie and Bylsma, 1928].

Animal	Body weight		Amount of plant fed	Remarks
	lb.	gm.		
Hill bull	218	400		Death within 1½ hours
Goat	54	20		10 gm. per day, death on the 2nd day
Sheep	71	10		Death after six hours

The writers found both the white and rose red varieties to be poisonous. This is in conformity with the observations of Wilson [quoted by Pammel, 1911].

The plant is considered to cause systolic heart failure in frogs, and Pendse and Dutt [1934] found that it contains a strong cardiac poison producing a depression of the heart.

ACTIVE PRINCIPLE

Greenish [1881 and 1883] isolated two bitter principles, namely, *neriodorin* and *neriodorein*. These toxic principles are possibly identical with *neriin* and *oleandrin* described by Schmidberg [1882 and 1883]. Bose [1901] isolated *karabin*. Pendse and Dutt [1934] made a systematic chemical examination and showed that the bark contained small quantities of a volatile essential oil, two amorphous glucosides, viz. *neriodorein* ($C_{23}H_{34}O_{11}$) and *neriodorin* ($C_{32}H_{52}O_7$), a solid crystalline wax, a phlobaphane, a tannin and a dark red colouring matter. The presence in the fresh bark of traces of peroxidase and hydrolytic enzyme has also been shown. Neumann and Linder [1937] have shown that the aglucone of oleandrin is identical with acetylgitoxigenin, the aglucone of desacetyl-oleandrin with gitoxigenin. They considered that chemically and pharmacologically both the glucosides belong to the group which may be designated as containing glucosides of digitalis group.

Fisher and Vidlerko [1937], however, found no alkaloids in the leaves, though Tschesche [1937] and Farbenind [1938] obtained glucosides from the leaves that have an action on the heart. Tschesche, Bohle and Neumann [1938] isolated the cardiac glucosides from oleander leaves and proposed the structural formula. Clinically, it is used like digitalis, but has produced better therapeutic effects.

SUMMARY

Observations were made on the toxicity of *Nerium odoratum* Fleanders to bulls, sheep and goats. It was found that all parts of the plant were highly toxic.

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AN EGG-COOLING CABINET

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(With Plate III and two text figures)

THE quality of an egg depends on inherited and environmental factors and the latter are more easily controlled than the former. Among the various external factors which influence egg quality temperature is normally the most important. Though eggs can be stored successfully for several months in cold storage, exposing them to considerably higher temperatures for even a comparatively short period will render them inedible. Ordinarily,

when subjected to high temperatures, eggs deteriorate rapidly, the air cell enlarges, the white liquefies and the yolk flattens out and ultimately ruptures.

Herrington and Sharp [1934] showed that, whilst the interior quality of eggs could at low temperatures, be conserved over fairly long periods, the rate of deterioration increased rapidly at higher holding temperatures. Eggs at 50°F. had their yolks in sound condition after 13 weeks

of storage, whereas others, held at 113°F., had ruptured yolks after storage for one week only.

The humidity of the atmosphere also influences egg quality. Very low humidity leads to rapid shrinkage, whilst very high humidity encourages mould growths. Both of these conditions are greatly accelerated if the temperature is high. Wilhelm [1939] reviewed the work carried out on the influence of temperature, humidity and other factors on egg quality. Kennard and Chamberlain [1940] and Klein [1940] showed that, even when temperatures were low, lack of humidity resulted in marked loss in grade as judged by candling. Shoemaker [1936] noted that high humidity in egg storage was essential for reducing evaporation and conserving flavour. The advantages of combining low temperatures with relatively high humidity in the storage of eggs have been demonstrated by Jeffrey and Darago [1940]; Benion and Price [1940]; Kennard and Chamberlain [1940]; *U. S. Egg and Poul. Mag.* [1940] and Snyder [1941].

Fertile eggs also become inedible as a result of embryo development after a few days' storage in a warm atmosphere. Rapid spoilage of eggs occurs in the plains of India owing to the fact that the temperature is often above the critical temperature for embryo development; indeed, in the hot season the temperature often approximates the optimum for incubation. Though the consumption of eggs in the towns is relatively much higher than in the villages, production is mainly confined to rural areas. The quality of the eggs found in the town markets, especially during the hot weather, is very poor on account of erratic collection from the producers, slow transport from the producer to the consumer and the exposure of the eggs at all stages to adverse weather conditions. As no attempt is made to produce infertile eggs at any season of the year, losses from embryo development during the hot months are excessively high.

The obvious remedy for the above losses would be to produce only infertile eggs during the hot weather and to hold them at low temperatures. In order to obtain infertile eggs, it would be necessary either to sell off the males or segregate them from the females. The latter remedy is, however, not very practical as production is much cheaper under free range conditions and it is not easy to keep the sexes separate in the villages where this system normally prevails. Furthermore, as the villager firmly believes that hens will stop laying or give very poor production in the absence of the male, it would require much propaganda to overcome the custom of running males with the females throughout the year. As proper cold storage conditions are altogether

impractical under present village conditions, it would be necessary to devise cheap and simple methods of storing eggs during the hotter seasons of the year. If such a procedure could be adopted, many eggs, which at present are a total loss, could be saved and consumption would be increased as the eggs would be of a better quality.

Various methods of storing eggs for short periods, in the absence of ice or mechanical refrigeration, have been tried. The *Canadian Poul. Review* [1940] quotes Cook as follows: 'In isolated cases we have found that the mean soil temperature during the summer months is generally low enough to suggest that a reasonably satisfactory storage could be provided in a suitable cellar with an insulated roof. In other districts the required low temperature might be obtained by evaporative cooling, i.e. the evaporation of water from moss peat, etc., placed around the cooled space. In other places sufficiently cool spring water might be available to cool a small holding room. Few of these methods could be capable of attaining temperatures of 50°F. but it should be possible to maintain 60-70°F. in most cases. The cellar system is useless where ground temperatures are high; evaporative cooling is useless where the relative humidity is high and the use of cold spring water, etc. is limited to the relatively small number of establishments where it is available'.

Payne [1934] gives the details of construction of an excellent type of underground cellar and reports that the inside temperature remained at 70°F. whilst the outside temperature was 100°F. This type of cellar is, however, too costly for the small producers and egg retailers normally found in India. Berley Winton [1933] gives details of another type which is only partially built into the earth, but it is unlikely that this system would prove as efficient. Collopy [1930], Dougherty [1931], *Canad. Poul. Rev.* [1940, 2] and Thompson and Roberts [1940] give details of several types of coolers based on the spontaneous evaporation of water. In northern India during the pre-monsoon months (April to June), the temperature is high and the humidity low, conditions which are favourable for cooling the air temperature by evaporating water.

EXPERIMENTAL

Fig. 1 and (Plate III, fig. 1) give details of a cooler similar to that described by Collopy [1930]. In order to ensure good aeration, the sides and bottom of the cabinet and the bottom of the trays were made of wire netting. Each of the trays held about 200 eggs. The cabinet gave the best results when exposed to a through draft in a shady place.

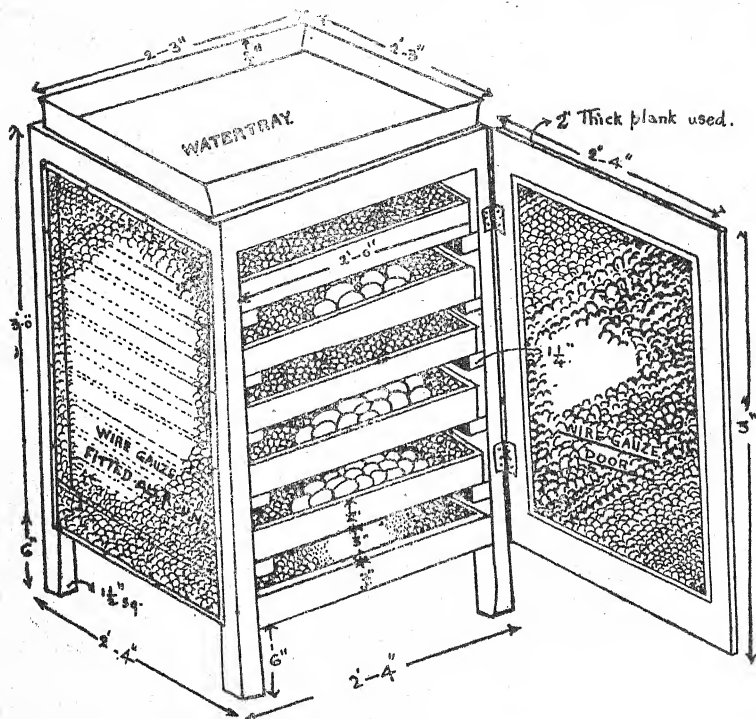


FIG. 1. Detail of construction of the cabinet cooler

To start the cooler, the water tray was filled with water, the hessian clothes were thoroughly soaked in water and hung over the cabinet with one end immersed in the water tray. The cabinet was not loaded with eggs until the temperature inside had ceased to fall. The cabinet was only opened when absolutely essential as frequent openings, especially during the heat of the day, increased the inside temperature and lowered the humidity.

Experiments were carried out to test the efficiency of the cabinet with regard to regulating the temperature and humidity and controlling embryo development, the extent of shrinkage and the deterioration of internal quality. In each test

similar groups of fertile and infertile, hour-old and day-old eggs were used. The quality of the hour-old, day-old and other eggs at various stages of storage both in the cabinet and under ordinary room condition were determined. Temperature and humidity records for the cabinet and room were maintained throughout each experiment.

TEMPERATURE AND HUMIDITY

Fig. 2 gives the results obtained in three experiments carried out in the hot dry period of May and June. The daily mean temperatures were obtained by averaging the daily maximum and minimum temperatures. The daily mean relative humidity percentages were obtained by

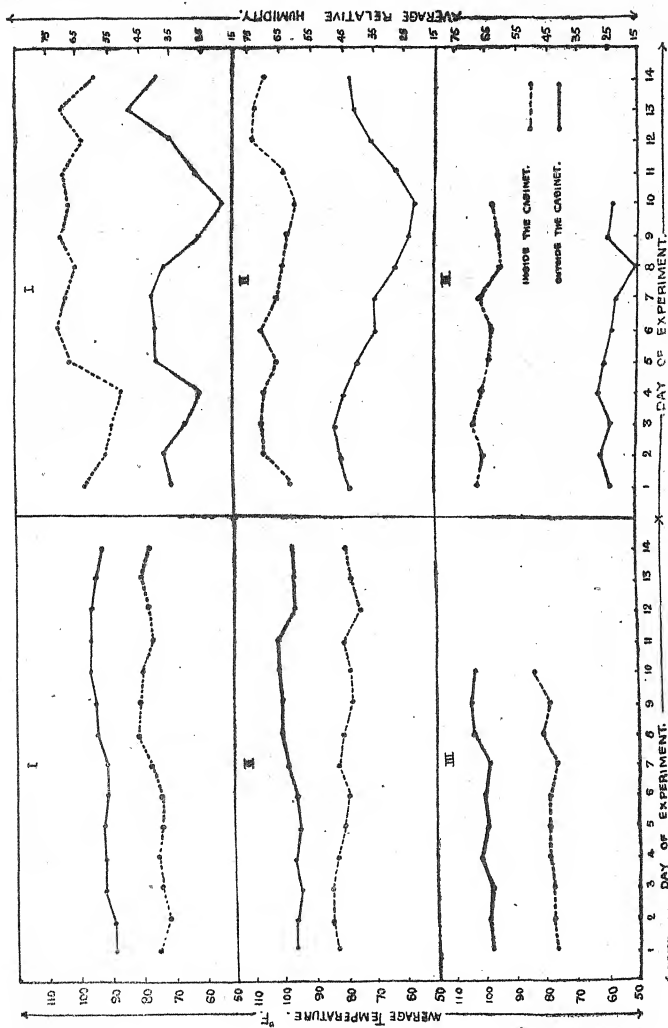


FIG. 2. Record of temperature and relative humidity inside and outside the Cabinet Cooler

averaging the morning and evening readings. The maximum daily temperatures recorded in the room ranged from 94° to 102°F. in Test 1, 98° to 106°F. in Test 2 and 100° to 110° F. in Test 3. The average room temperatures for each of the tests were 93.1°, 97.3° and 100.3°F., in Tests 1 to 3 respectively. The corresponding temperatures inside the cooler were 76.7°, 80.2° and 78.7°F. respectively. Mean relative humidity percentages in the room in Tests 1 to 3 were 34, 36 and 23; the corresponding figures in the cabinet were 65, 67 and 63 respectively.

In all the three experiments the cabinet temperatures were much lower than those recorded for the room. In Test 3 the average drop in temperature was greater than in Tests 1 and 2 owing to the higher room temperature and lower humidity, both factors being more favourable for the efficient working of the cooler. A maximum drop in temperature of 36°F. was recorded during a period of strong hot winds when the room temperature was 110°F. and the relative humidity below 5 per cent. Thompson and Roberts [1940] obtained a maximum drop of 24°F. with a temperature of 103°F. and a relative humidity of 9 per cent.

In preliminary experiments it was found that the conditions of fairly high temperature and humidity inside the cabinet favoured mould growth in eggs stored for more than ten days. In a number of experiments it was found that whilst all the eggs remained apparently free from mould during the first 10 days of storage, further storage for four to five days resulted in nearly all of them developing mould growths. This defect in storage under cabinet conditions was overcome by transferring the eggs into a perfectly fresh cabinet after one week's storage. The cabinet after use for one week was thoroughly washed and left to stand in the sun for a period of three to four hours and rested until required again for use at the end of a week. With two cabinets, one in use for one week and the other rested after cleaning for one week, no trouble was experienced with mould growths in various tests involving storage for four weeks in the cabinets.

EMBRYO DEVELOPMENT

A total of 240 fertile eggs were used to test out the efficiency of the egg cabinet in retarding embryo development. The maximum daily room temperatures recorded during the storage period ranged from 103° to 110°F. Hour-old eggs and day-old eggs that had been stored at room temperature were used and stored either in the cooler or in the room. The hour-old eggs would normally only be available with the producer who collects the eggs every hour. The day-old eggs represent the very best quality eggs as received by the retailer or consumer. The details of the tests carried out at different stages are given in Table I.

TABLE I
Embryo development

Period of storage	Hour-old eggs stored in		Day-old eggs stored in		Remarks
	Cooler	Room	Cooler	Room	
Fresh	+	+	No. 35
3 days	..	+	+	+++	No. 35
7 days	..	++++	++	++++	No. 35
10 days	..*	++++	++	++++	No. 35
14 days	..*	++++	++	++++	No. 35

+ Development without blood, visible clearly on candling.

++ Development with trace of blood, visible only on opening out.

+++ Further development with blood veins clearly visible on candling.

++++ Extensive development.

*Slight germ development without blood, visible only on opening out.

Hour-old eggs stored at room temperature for three days were classified as edible. They showed prominent embryo development but no blood under the candling lamp. Hour-old eggs stored at room temperature for seven days or more were judged as inedible on account of extensive embryo development.

Under the cabinet condition of storage the eggs showed no signs of embryo development under the candling lamp at 10 and 14 days. However, at 10 and 14 days about 50 per cent of the eggs when broken showed very slight embryo development. The embryo development in eggs stored for 14 days in the cabinet was much less than that of similar eggs stored for three days at room temperature.

The day-old eggs stored at room temperature showed a certain amount of embryo development under the candling lamp but no blood was visible. The eggs when stored at room temperature for a further period of three days showed marked embryo and blood development under the candling lamp. These eggs were classified as inedible. At each of the subsequent stages of holding at room temperature the quality was somewhat poorer than the corresponding hour-old eggs stored under the same conditions. The day-old eggs stored in the cabinet for three days showed slightly greater embryo development than at a day-old but were still edible as no blood had formed. After 7, 10 and 14 days in the cabinet the day-old eggs showed slightly more marked development of the embryo under the candling lamp than at three days but no blood was visible. However, at all these three later stages the broken out eggs had very minute

specks of blood. From the market standpoint such eggs, though not of the highest quality, can be considered as edible.

SHRINKAGE

Shrinkage in eggs is brought about by the loss of moisture and is regulated by the temperature and humidity of the atmosphere. In commercial marketing it is impossible to determine the shrinkage direct, but an indirect measurement of shrinkage can be made by studying the size of the air space as seen by means of the candling lamp. The maximum permissible air cell depth for Agmark as laid down in the Agricultural Produce (Grading and Marking) Act (1937) is three-eighths of an inch [Report on Marketing, 1938]. As judgment of the air cell depth under the candling lamp is somewhat empirical, 240 fertile and 240 infertile eggs were broken and the size of the air cell measured by means of a spherometer (Plate III, Fig. 2). The various measurements for the depth of the air cell and the percentage losses of weight from the original weights as measured by actual weighings are recorded in Table II.

TABLE II
Shrinkage

Period of storage	Place of storage	Hour-old eggs		Day-old eggs	
		Depth of air cell mm.	Loss of weight per cent	Depth of air cell mm.	Loss of weight per cent
Fresh	...	1.1	...	4.3	...
3 days	Cooler	4.9	0.78	5.2	0.55
	Room	6.1	1.76	7.0	1.68
7 days	Cooler	6.3	1.91	6.8	1.59
	Room	8.6	3.99	9.6	4.61
10 days	Cooler	7.1	2.54	7.3	2.14
	Room	9.9	5.78	11.2	6.94
14 days	Cooler	7.6	3.39	7.8	2.92
	Room	12.5	9.24	13.2	9.90

At hour-old the average depth of the air cell was only 1.1 mm. whereas in day-old eggs stored under room conditions the corresponding figure was 4.3 mm. In all cases the depth of the air cell increased with storage; the figures for the hour-old and day-old eggs held under room conditions for 14 days were 12.5 and 13.2 mm. respectively. The increase in the depth of the air cell under cabinet conditions was much less and after 14 days of storage the corresponding figures were 7.6 and 7.8 mm. for the hour-old and day-old eggs respectively.

The percentage loss of weight followed much the same trend as the air cell depth for at each stage the hour-old and day-old eggs in the cabinet had shrunk less than those under room conditions. At 14 days storage the percentage losses in weight in the hour-and day-old eggs were 9.24 and 9.90

whilst the corresponding figures for the cabinet stored eggs were only 3.39 and 2.92 respectively.

Shell thickness determinations were made on all the stored eggs in order to find out if there was any correlation between the thickness of the shell and the percentage loss of weight. Micrometer screw gauge readings of the thickness of the shell were made on each egg at each pole and at a point midway between the poles and these figures were averaged to get the shell thickness. A critical examination of the results obtained revealed no close relationship between the shell thickness and percentage loss of weight with shells ranging in thickness from 0.22 to 0.45 mm.

Table III gives comparative figures for the percentage losses in weight for a dozen selected pairs of eggs. Both eggs in each pair were treated identically. The figures showed that the percentage loss in weight is not correlated with the thickness of the shell. This is in agreement with the findings of Romanoff [1937].

TABLE III

Relation of shell thickness to percentage loss in weight

No.	Shell thickness mm.	Loss of weight per cent
1	0.26	1.57
	0.26	3.02
2	0.27	5.42
	0.27	7.62
3	0.29	6.21
	0.30	7.62
4	0.31	9.28
	0.32	13.00
5	0.33	4.29
	0.34	9.06
6	0.34	2.90
	0.34	4.94
7	0.34	1.22
	0.35	2.10
8	0.34	4.87
	0.36	9.79
9	0.34	4.39
	0.38	10.04
10	0.36	5.65
	0.43	8.65
11	0.37	3.55
	0.38	6.39
12	0.38	4.58
	0.38	10.58

INTERIOR QUALITY

The question of the interior quality of the yolk and white, apart from embryo development, was also investigated. The quality factors studied were albumen index, percentage thick white and yolk index as described by Macdonald and Krishnan [1942, 1943]. Table IV gives data for hour-old eggs stored in May and Table V gives data for day-old eggs stored in June. As the average temperatures both in the room and cabinet were somewhat higher in June than in May, the two tables cannot be regarded as strictly comparable.

Under room conditions the yolk index figures for the fertile hour-old eggs decreased from an initial 0.433 to 0.229 at seven days. During the same period the rate of decline in the infertile eggs also was very similar. From seven days onward the rate of decline in the yolk index figures was greater in the case of the fertile than the infertile eggs. Whilst it was possible to make yolk index determinations on all the samples of infertile eggs stored at room conditions for 10 and 14 days, it was only possible to make two determinations in the case of the fertiles at 10 days and none at 14 days. The yolk index figures for the fertile and infertile eggs were fairly comparable under cooler conditions. Yolk index determinations were made on all 12 samples of fertile eggs held in the cooler for 14 days. The yolk index figures at each stage of storage were higher in the cooler than in the room.

In the case of the day-old eggs the yolk index figures were similar for fertile and infertile eggs held for three days under room conditions. However at

seven days and at each subsequent period under room conditions all the fertile eggs had burst yolks whereas it was possible to record all the yolk index figures of 7 and 10 days and 8 out of 12 at 14 days of the infertiles held under the same conditions.

As in the case of the hour-old eggs the day-old eggs stored under cooler conditions gave better results as judged by yolk index than the eggs stored under room conditions. In the case of fertile eggs it was possible to determine the yolk index of all eggs at seven days in the cooler whereas no readings were possible at this stage on fertiles held under room conditions.

The albumen quality factors, viz. albumen index and percentage thick white show that the conditions in the cooler were not so favourable to their preservation as in the case of the yolk index. There was also less difference in the behaviour of the fertile and infertile eggs both under room and cooler conditions of storage. Readings for the albumen index were often impossible on account of rupture of the thick white layer. Rupture of the yolk in fertile eggs also seriously interfered with the recording of data. The thick albumen layer was ruptured in nearly all the fertile eggs at three days in the day-old lot and at seven days in the hour-old lot, when held at room temperature. The fertile eggs in the cooler had the thick albumen intact for about 3 days longer than those in the room. Under the cabinet conditions the albumen index figures were somewhat better than those obtained under room conditions.

TABLE IV

Interior egg quality—hour-old eggs

Period of storage	Place of storage	Yolk index		Albumen index		Percentage thick white	
		Fertile	Infertile	Fertile	Infertile	Fertile	Infertile
Fresh	..	0.433	0.433	0.096	0.103	63.5	65.0
3 days	Cooler	0.386	0.401	0.056	0.048	53.8	52.4
	Room	0.349	0.330	0.041	0.044	54.1	51.8
7 days	Cooler	0.314	0.350	(6) 0.050	(7) 0.036	47.8	52.8
	Room	0.229	0.234	(1) 0.025	(5) 0.032	52.1	52.4
10 days	Cooler	0.284	0.292	(4) 0.036	(7) 0.032	49.6	47.7
	Room	(2) 0.140	0.179	..	(4) 0.030	..	46.4
14 days	Cooler	0.239	0.271	(2) 0.024	(2) 0.034	39.9	42.4
	Room	..	0.165	..	(1) 0.046	..	40.8

TABLE V

Interior egg quality—day-old eggs

Period of storage	Place of storage	Yolk Index		Albumen Index		Percentage thick white	
		Fertile	Infertile	Fertile	Infertile	Fertile	Infertile
Fresh	0.382	0.397	0.056	0.060	48.5	49.3
3 days . . .	Cooler . . .	0.330	0.330	0.041	0.041	54.1	46.7
	Room . . .	0.257	0.260	(2) 0.024	0.039	49.5	50.4
7 days . . .	Cooler . . .	0.283	0.264	(7) 0.035	0.031	56.7	47.1
	Room	0.176	..	(5) 0.034	..	52.1
10 days . . .	Cooler . . .	(6) 0.264	0.227	(2) 0.036	(5) 0.035	(6) 47.8	44.8
	Room	0.151	..	(2) 0.028	..	46.0
14 days . . .	Cooler . . .	(6) 0.252	0.212	(1) 0.025	(3) 0.029	(5) 43.6	42.3
	Room	(9) 0.132	30.6

N.B. The quality factor figures for the fresh eggs are the average of 24 samples. The numbers in brackets above the figures indicate the number of samples remaining fit for examination. The other figures represent the average of 12 samples.

The percentage thick white figures indicate that there is a very marked loss in quality during the first 24 hours and that the decline thereafter is relatively slow. Thick white measurements could not be carried out at certain stages on account of the rupture of the yolk. As in the case of the albumen index figures the results indicate that the quality of the white is not very materially improved by storing the eggs under cooler conditions.

SUMMARY

Details are given in regard to the construction and efficiency of a simple type of cabinet, wherein the temperature was reduced by means of evaporating water. During the hot dry period preceding the monsoon when the average room temperature was around 100°F. and the relative humidity was below or around 30 per cent, the temperature inside the cabinet was about 20°F. lower and the relative humidity 30 to 40 units higher than that recorded in the room. Storage inside the cabinet proved of very material benefit in reducing shrinkage in eggs as judged by size of the air space and the percentage loss in weight, and in improving egg quality as judged by candling and actual measurement of the interior quality. Holding eggs in the cooler did not very materially improve the keeping quality of the white as judged by albumen index and percentage thick white. However, eggs stored in the cabinet maintained markedly better yolk conditions than those held under room conditions. Maximum benefit from the cabinet was obtained with fertile eggs which remained edible for 14 days under cabinet storage,

whilst similar eggs under room conditions became inedible inside seven days on account of embryo development.

Data collected confirm the finding that the thickness of the egg shell does not materially influence the rate of shrinkage in eggs.

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A CASE OF PSEUDOTUBERCULOSIS (*PASTEURILLA*) PSEUDOTUBERCULOSIS INFECTION) IN THE GOAT

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(With Plates IV and V)

LESIONS somewhat resembling those of tuberculosis may result from a variety of causes, viz. coccidiosis, strongylosis and cysticercus infestation; bacteria such as *Pasteurella pseudotuberculosis*, *Corynebacterium ovis*, *C. murium* and *C. equi*, may also cause similar lesions. Before Koch introduced the plate technique of isolating pure cultures of bacteria and elucidated the true cause of the tuberculosis, there is no doubt that several of the above conditions were often confused with tuberculosis.

Malassez and Vignal [1883] are considered to be the first to recognize the infection with *Pasteurella pseudotuberculosis*. They described a disease in a guinea-pig which had been experimentally inoculated with material from a subcutaneous nodule in the arm of a child who died of tubercular meningitis. The resulting lesions resembled tuberculosis to the naked eye, but instead of the tubercle bacillus zoogloal masses of coccoid bodies were found in them. They called the disease zoogloal tuberculosis. Between 1883 and 1894 several workers described diseases occurring naturally in several species of animals, particularly rodents, and they believed they were dealing with the same disease as that of Malassez and Vignal. The terms pseudotuberculosis, zoogloal tuberculosis, bacillary pseudotuberculosis and tuberculoform nodules were freely used in describing the disease and in describing the resulting lesions. Some workers loosely referred to giant cell-like formations in these lesions, while others emphasized the rarity or absence of giant cells. The descriptions, particularly of the causal organisms, were inadequate. There was confusion in the picture presented, and the matter undoubtedly required clarification, in view of the fact that Preisz and Guinard [1891] had in the meantime described a disease in sheep, caseous lymphadenitis, which they also called pseudotuberculosis.

Working towards this end, Preisz [1894] made a comparative study of all the available strains which had been isolated from cases of pseudotuberculosis. He came to the conclusion that the same microbe was responsible for the several cases of pseudotuberculosis in rodents. He called the disease 'pseudotuberculosis rodentium' and for the parasite he adopted the name, *Streptobacillus pseudotuberculosis*. The ovine disease was caused by a different bacterium, which he designated as pseudotuberculosis ovis.

Pasteurella pseudotuberculosis infection is now widely recognized as a common cause of an epidemic disease among rodents. The disease occurs in guinea-pigs, rabbits, hares and musk rats. Within the last decade the prevalence of the disease among birds has been gradually established. It has been reported in fowls, turkeys, pigeons, pheasants, in a black-bird and in canaries. Several cases of the disease in man are also on record. Sporadic cases have been encountered in a variety of other higher animals, such as the cow, horse, cat, pig, monkey, goat and roe-deer. Though the disease has been reported in the higher animals, it will be seen that except perhaps in man such animals are only occasionally affected. The relatively high incidence in the cat is due to the fact that cats in nature prey upon rodents. There is only a single record of the incidence of the disease in goats [Baumann, 1927]. We are, therefore, recording in some detail a case of pseudotuberculosis encountered by us in a goat.

CLINICAL HISTORY

The case under reference was one of 49 goats purchased on 9 December 1940 for rinderpest virus production. It is customary to record the temperature of these goats for some days before they are brought under experiment. The temperatures of the batch to which this goat (No. 374) belonged were recorded from the date of purchase. On 4 January 1941, it was reported that this animal's temperature was 104.9°F. On examination, signs of pneumonia were noted. The temperature remained high for 11 days. During this period the animal became progressively weaker and more emaciated. The temperature gradually declined between the 11th and 15th day and before death on the 17th day was subnormal, and the animal was recumbent.

POST MORTEM FINDINGS

Changes were found in the lungs and the liver. The former were studded with greyish-white nodules resembling tubercles, distributed evenly throughout the substance, each being about the size of a millet seed and shot-like in consistency (Plate IV, fig. 1). The superficial nodules were surrounded by areas of congestion and consisted of a hard caseating mass, without any evidence of calcification.

The liver also presented nodules of about the same size and type, evenly distributed throughout

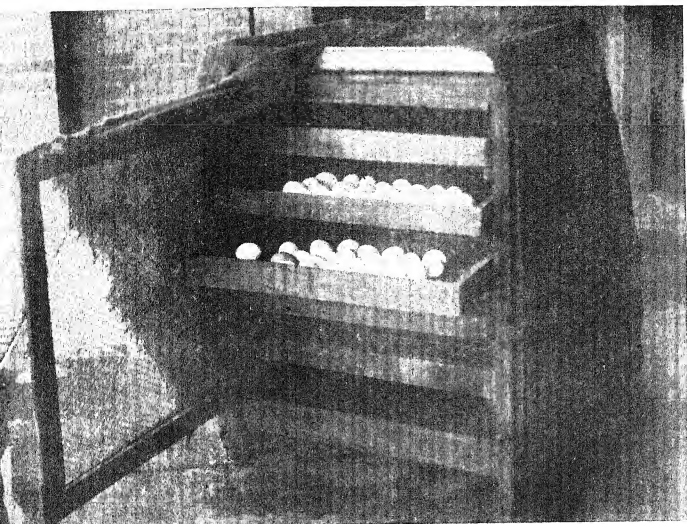


FIG. 1. The Cooler in use

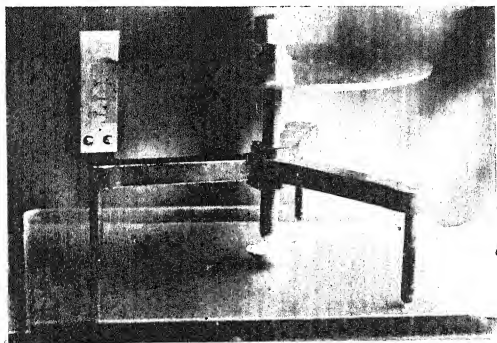


FIG. 2. Measurement of depth of air-cell



FIG. 1. Lung lesions in goat No. 374

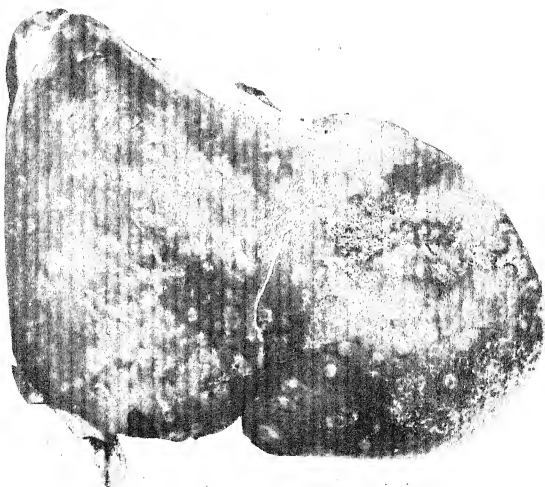


FIG. 2. Lesions in the liver of goat No. 374

the substance, with a tendency to project above the surface (Plate IV, fig. 2). The liver was somewhat tougher than normal.

The right kidney was enlarged. The mediastinal, portal and mesenteric glands were enlarged but presented no evidence of caseation.

It is important to note that the nodules were evenly distributed throughout the substance of organs affected, whereas Baumann [1927] records that in his case the lesions were confined to the surface of the organs.

HISTOPATHOLOGY

The most prominent and characteristic lesion was the formation of granulomata in the liver and the lungs. The histological structure and development of these pseudotubercles were best appreciated in the sections of the liver.

The youngest nodules, visible only under the microscope, were represented by focal collections, in the interstitial tissue, of mononuclear cells, mainly lymphatic and partly monocyteic in type (Plate V, fig. 1). With the growth in size of these cellular collections, necrotic changes set in quite early, resulting in the loss of cell outline and marked by extensive karyorrhexis of the inflammatory cells. Old macroscopic nodules were composed almost entirely of a mass of nuclear debris lying in a practically structureless bed surrounded by a narrow zone composed of morphologically intact polyblasts and, towards the periphery, by a few fibroblasts, with evidence (in the form of comparatively scanty collagen tissue) of ill-defined encapsulation (Plate V, fig. 2). Towards the centre of the oldest and largest nodules were seen several areas which, in contrast with the surrounding nuclear detritus, were completely amorphous, took up the eosin stain and in all probability were evidence of caseation. It was also in the largest nodules that fibrous tissue formation was more evident.

Giant cells were absent, while neutrophil and eosinophil cells were never present in significant numbers. There was no evidence of club-formation as seen in 'actinophytotic' granulomata.

As a rule, the nodules were comparatively avascular, but in a few large ones fair sized healthy looking blood vessels had persisted. It was towards the periphery of the nodules, however, that several apparently newly-formed blood and bile vessels were present. The surrounding parenchymatous tissue showed ample evidence of compression. The adjacent columns of liver cells were much distorted, and the individual liver cells atrophied. The sinusoidal spaces were greatly widened and showed a more or less generalized infiltration of inflammatory mononuclear cells.

There was also some cellular infiltration in Glisson's capsule, but cirrhosis was not a prominent feature. Some bile ducts showed catarrhal changes and were denuded of epithelial lining. There were a few haemorrhages, but no evidence of pigmentation.

The pulmonary lesions presented essentially the same features as those seen in the liver and were primarily of the nature of an interstitial pneumonia. Besides several well-defined granulomatous nodules of various sizes, the lungs showed a general congestion of blood vessels, a low grade bronchial catarrh, accompanied by a more or less generalized cellular infiltration in the peribronchial and perivascular interstitial tissue and a catarrhal pneumonic change, and consolidation of the alveoli surrounding the nodules. The alveolar exudate was highly cellular, and as one would expect in pathological processes of this type, was composed mainly of catarrhal epithelial cells and mononuclear polyblasts. As in the liver, the polymorphonuclear cells were scanty, and fibrin formation was not marked. The predominance of lymphocytes in the contents of the larger blood vessels, along with other available evidence, clearly indicated that these cells were primarily concerned in this pathological process.

No gram-positive or acid-fast organisms were found but sections of the liver or lung stained with methylene blue or Leishman's stain revealed numerous clusters of round and ovoid microorganisms. There was no histological evidence of worm infestation nor were any worms or their developmental stages seen in the sections examined.

BACTERIOLOGICAL EXAMINATION

Pure cultures of the organism were obtained from the spleen, liver, lungs and some of the enlarged lymphatic glands.

The organism was Gram-negative, non-sporing and seen in films as short rods. It was non-motile when grown at 37°C., but actively motile when grown at room temperature (18°C.). On agar plates it formed bluish-grey, relatively opaque flat colonies, with entire edge and shining moist surface. In broth there was uniform turbidity, with a tendency for the growth to form thin, loose flakes at the surface which fell to the bottom on disturbing the tube. There was a powdery deposit, which increased in 48 hours, at the cost of the turbidity of the supernatant fluid. The organism formed acid but no gas from dextrose, maltose, mannitol and salicin. Lactose and sucrose were not attacked. It did not liquify gelatin, formed no indol, did not reduce nitrates, produced hydrogen sulphide, and rendered litmus

milk slightly alkaline. It was V. P.— and M. R. +. The organism is a member of the *Pasteurella pseudotuberculosis* group.

Agglutination tests were conducted to ascertain to which of Schütze's serological group the organism belonged. Type strains belonging to groups I, II, III and IV were included. An antiserum made in the rabbit against flagellated goat strain organism agglutinated H—suspensions of the homologous organism and of all the type strains

except the one belonging to Group III. O—antigens had a tendency to spontaneous settling. Slide agglutination tests were, therefore, conducted with O—antigens made from agar growths. The serum agglutinated the homologous antigen and among the type strains only those belonging to group I. The organism belongs to Schütze's serological group I (Table I.) No absorption test was done, and the sub-type has not been determined.

TABLE I

Agglutination reaction obtained with H+O serum made against the organism obtained from the goat titre

N. C. T. C. No. and serological type		No. 1102 Gr. I, Type A	No. 824 Gr. I, Type B	No. 2476 Gr. II, Type A	No. 1779 Gr. II, Type	No. 3245 Gr. III	No. 3570 Gr. IV	Homologous strain
Antigen	'H'-anti- gen (tube test)	800	1,600	400	400	..	800	1,600
	'O'-anti- gen (slide agglutination test)	+++	+++	—	—	—	—	+++

* +++ = Distinct clumping within one minute.

— = No clumping in five minutes.

PATHOGENICITY TESTS

A broth culture of the organism was injected intravenously at a dose of 0.2 c.c. into a rabbit. The animal died six days later and revealed on autopsy lesions resembling those of miliary tuberculosis, chiefly in the lung, liver and spleen. *Pasteurella pseudotuberculosis* was re-isolated in pure culture from the affected organs.

Another rabbit was under immunization with carbolized cultures of the organism for the production of agglutinating serum. After a course of 12 injections, the animal received a dose of 0.3 c.c. of living broth culture and died six days later. On autopsy, it revealed lesions similar to those of the first rabbit, save that in this case the liver was more extensively involved than the lungs and the spleen.

Four goats were infected, two by the subcutaneous inoculation of 1.0 c.c. of an 18-hours broth culture and two by an intranasal spray of the same amount of culture. The goats died of pneumonia at intervals ranging from eight to 20 days. *Post mortem* examination revealed on

other abnormality than consolidation of the greater part of the anterior lobes of the lungs. No nodules were seen by the naked eye, but sections revealed the presence of early nodules evidenced by the accumulation of lymphoid cells. *Pasteurella pseudotuberculosis* was re-isolated from the spleen and the consolidated portions of the lungs.

SUMMARY

A description is given of a case of pseudotuberculosis in a goat which appears to be the second case so far reported in this species. The symptoms, gross morbid anatomy and histopathology of the condition are described. The causal organism was identified as *Pasteurella pseudotuberculosis*, group I (Schütze).

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COLOURING SEPARATED MILK TO PREVENT ITS USE FOR ADULTERATING WHOLE MILK

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(With two text-figures)

Milk obtained from Indian cows and buffaloes is rich in fat. The usual legal limits prescribed for fat in most of the provinces are very low. This provides a great scope for practising adulteration by the simple manipulation of fat content and specific gravity. When separated milk is added even the chemical tests may fail. Under these circumstances a consumer is hardly able to judge the quality of his milk supply.

It is, therefore, proposed that all the separated milk to be consumed in urban centres should be distinctly coloured, so that even when small quantities of colouring matter are added to milk, its presence can be detected visually. For this the colour chosen must naturally be attractive, and it is thought that a light pink colour will best serve this purpose. Similar devices have been employed in other countries in the past to encourage milk drinking habits among school children.

The following study was carried out to find a suitable dye and to examine its stability under trade conditions.

Selection of dyes. Observations were made with (i) Edicol erythrosine, (ii) eosine, (iii) congo red, (iv) cochineal extract and (v) beetroot extract. All these colours are soluble in water.

The cochineal extract used in the present experiments was one made by Messrs J. N. Nichols & Co. Ltd., Manchester.

The beetroot extract was obtained by pulping raw beetroots without any addition of water. The extract was filtered and preserved in cold storage.

Safranine was also found to impart the desired colour to milk, but it is known to be poisonous and hence no further trials were carried out.

Concentration of dyes. The minimum concentration of dyes to be used for colouring separated milk is shown in Table I. Such concentrations are sufficient to permit visual detection of mixtures of as little as 5 per cent coloured separated milk with whole milk.

TABLE I
Concentration of dyes

Dye	Minimum concentration	
	A In ordinary light	B In artificial light
Edicol erythrosine . . .	0.012 per cent (gm./100 ml.)	0.008 per cent (gm./100 ml.)
Eosine	0.008 per cent (gm./100 ml.)	0.0008 per cent (gm./100 ml.)
Congo red	0.006 per cent (gm./100 ml.)	0.0015 per cent (gm./100 ml.)
Cochineal extract . . .	0.3 (ml./100 ml.)	0.04 (ml./100 ml.)
Beetroot extract . . .	1.2 (ml./100 ml.)	0.60 (ml./100 ml.)

Physical properties of coloured separated milk. The effects of various conditions on the coloured separated milk, which are likely to be encountered under marketing conditions, were examined.

(a) **Taste of coloured separated milk.** Samples of separated milk were prepared for each dye, using the concentration mentioned above in column A. A control uncoloured sample was also kept for comparison. Judging for taste and flavour was carried out independently by several persons at intervals. The average results are shown in Table II. The use of the first four dyes listed above seems to mask the raw, normal flavour of milk and thus improve its taste. Beetroot extract on the other hand imparts a foreign flavour, possibly due to the large concentration in which it was used.

(b) **Effect of exposure to sunlight.** Samples of coloured separated milk were kept in direct sunlight, together with the control for six hours. It was found that there was no change in the quality of treated samples as compared to the control. All the coloured samples retained their original colour. This was judged by comparing the colour with a similar lot which was kept in the dark.

Colouring Separated Milk

TABLE II

Effects of dyes on the flavour and taste of separated milk

Particulars of sample	Average score				
	0 hour	2 hours	4 hours	6 hours	7 hours
Control sample	++	++	++	++	++
Coloured with Edicol erythrosine	+++	+++	+++	+++	+++
Coloured with eosine	+++	+++	+++	+++	+++
Coloured with congo red	+++	+++	+++	+++	+++
Coloured with cochineal extract	+++	+++	+++	+++	+++
Coloured with beetroot extract.	+	+	+	+	+

(c) *Effect of storage at room temperature.* Samples of separated milk coloured with eosine, congo red, beetroot extract and cochineal extract were not found to fade or give any precipitate when stored till they curdled.

Edicol erythrosine showed a noticeable tendency to precipitate out, after standing over four hours.

(d) *Effect of storage under refrigeration.* Samples of separated milk coloured with eosine, congo red and cochineal extract preserved their freshness and colour when stored for 24 hours at 45°F.

The sample coloured with Edicol erythrosine gave a precipitate, as also the one coloured with beetroot extract. In the latter case the colour of the precipitate was dirty white.

(e) *Effect of boiling.* When first brought to

a boil the samples coloured with eosine and Edicol erythrosine showed no change.

In the case of congo red, the colour became more intense, whereas that of cochineal extract faded slightly and that of beetroot extract faded markedly.

(f) *Use of coloured milk for making curd.* There was no difference in the appearance and flavour of curd obtained from the control sample and those from the coloured samples.

(g) *Effect of storing coloured milk in different containers.* Coloured and uncoloured samples were stored simultaneously in containers made of different material and tested for flavour at intervals of two hours over a period of eight hours by different judges independently. The average score is shown in Table III.

TABLE III

Effect of storing milk in different types of containers

Containers used	Dye used									
	Edicol erythrosine		Eosine		Congo red		Cochineal extract		Beetroot extract	
	Control	Coloured	Control	Coloured	Control	Coloured	Control	Coloured	Control	Coloured
Glass bottles	++	+++	++	+++	++	++	++	++	++	+
Tinned steel cans	++	+++	++	+++	++	++	++	++	++	+
Aluminium cans	++	+++	++	+++	++	++	++	++	++	+
Kalattad brass cans	++	+++	++	+++	++	++	++	++	++	+

Detection of adulteration of genuine milk with coloured separated milk. When separated milk coloured with various dyes, in the concentrations indicated in column A, Table I, was mixed with cow or buffalo milk, adulteration could be easily detected by sight to the extent of five per cent and more. With lower concentrations of the dye the indication was not so definite.

The sensitivity of the test can, however, be

greatly increased by examining the sample in artificial light. For this purpose, the device shown in Fig. 1 is used. It consists of a wooden box with two holes. The sides of these holes are covered with rubber washer. Under each of the holes there is an electric bulb (40W). On one hole a control sample of milk contained in a glass vessel is placed, while over the other one is placed a sample that is suspected to contain coloured

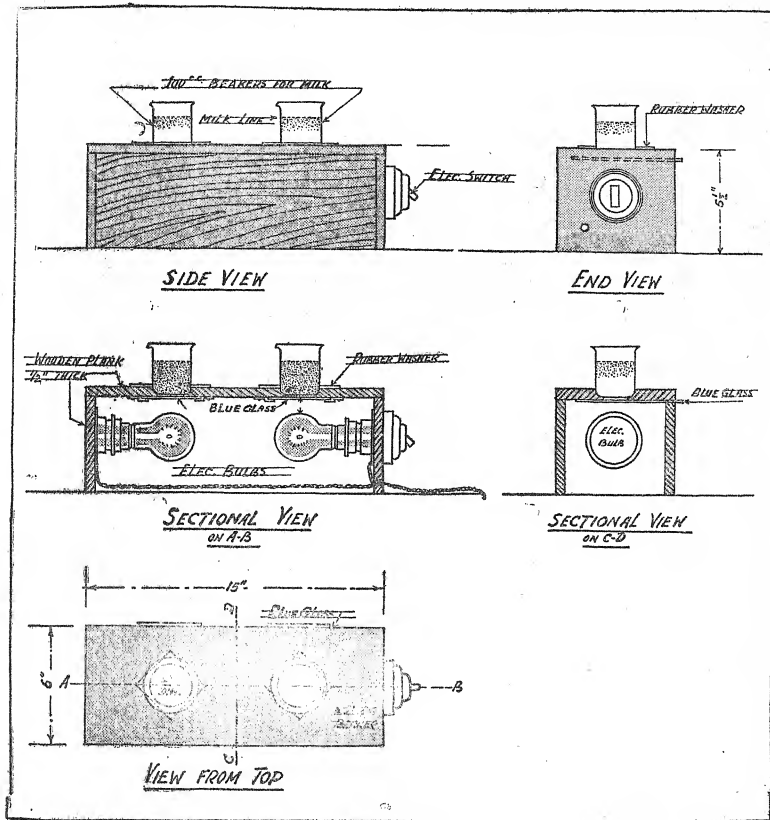


FIG. 1. Device for detecting coloured separated milk

separated milk. When the lights are switched on, the adulterated samples appear distinctly red, while the unadulterated samples show only a white colour. By the help of this simple device the amount of dye actually required for colouring separated milk is very small in each case and corresponds to the quantities given under column B in Table I.

The sensitivity of the test varies with the intensity of artificial light. A 40 W bulb was found suitable. It was found that a light blue coloured (day-light lamp) bulb was preferable to a plain one.

On a field scale when an electric supply is not always available, ordinary torch cells with a tiny

blue bulb may be used. The bulb is dipped directly in the milk or is placed in a test-tube, which is

then dipped in the milk container. This is illustrated in Figs. II (A) and II (B).

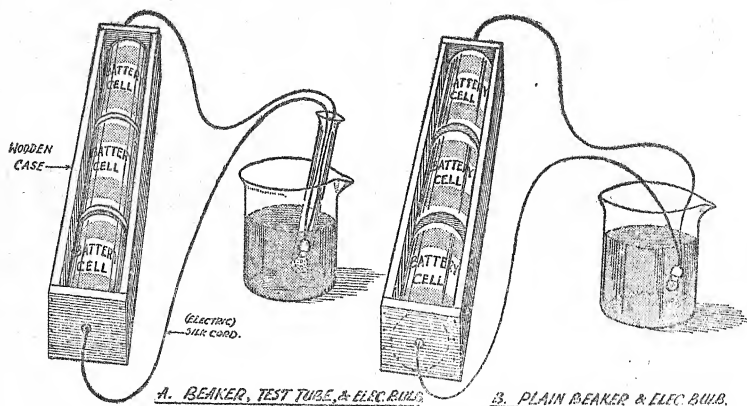


FIG. 2. Field outfit for detecting presence of coloured separated milk

SUMMARY

To prevent the adulteration of milk with separated milk, colouring of the latter to a pink colour is suggested. For this purpose eosine, congo-red, Edicol erythrosine, cochineal extract and beetroot extract were tried. Coloured milks were studied under various conditions likely to be encountered by the trade. These studies show that eosine and congored are the most suitable

dyes. Next to them come erythrosine and cochineal extract. Beetroot extract is not suitable, as it imparts an objectionable flavour to the coloured milk.

A simple device is described for viewing the samples of milk adulterated with coloured separated milk under artificial light. By the use of this apparatus the concentration of dyes to be used is greatly reduced, and it is still possible to detect as little as 5 per cent adulteration.

INVESTIGATIONS ON FAMINE RATIONS FOR LIVESTOCK

I. MUNJ AND MOLASSES AS FAMINE RATIONS

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(Received for publication on 4 June 1943)

(With Plate VI and two text-figures)

THE irregularity of rainfall in certain tracts of India is solely responsible for periodic famines, and the question of feeding stock during these times is of paramount importance.

In examining the fodder resources of the country for the cattle population, Wright [1937] has arrived at approximate figures which are given in Table I.

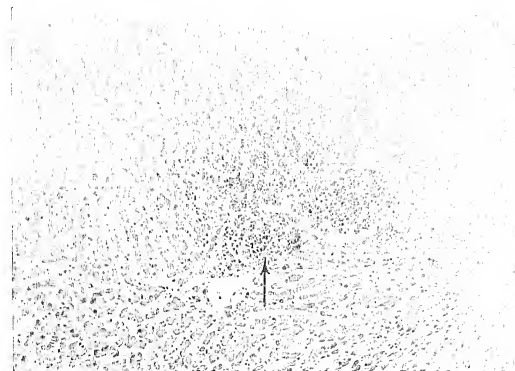


FIG. 1. Liver nodule. Early stage $\times 140$.
Some of the youngest nodules
represented by focal collection of
mononuclear cells.

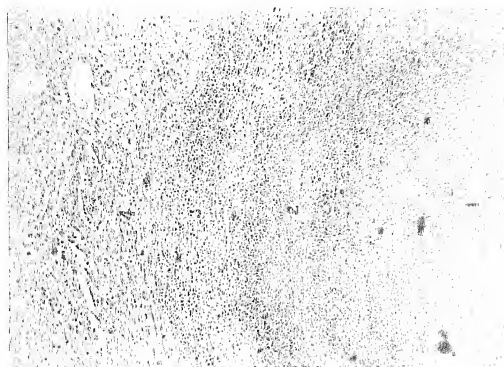


FIG. 2. Liver nodule. Late stage $\times 70$.
1. Completely amorphous eosinophilic
centre
2. Area composed of nuclear detritus
3. Zone of intact polyblasts
4. Compressed liver cells at the junction
of healthy and diseased tissue



FIG. 1



FIG. 2



FIG. 3

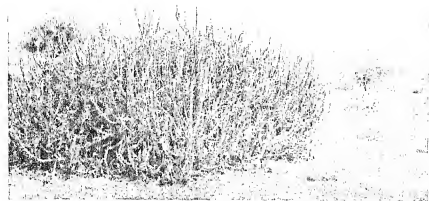


FIG. 4



FIG 5

FIGS. 1 & 2. Sheep, goats and camels appear happy as compared with cattle and seem able to subsist on the flora available in the xerophytic areas.

FIG. 3. The last few leaves on *Kikar* being lopped for feeding hungry livestock.

FIGS. 4 & 5. Typically xerophytic plants which are either toxic or bear tough thorns are the only survivors in the sunburnt overgrazed areas.

TABLE I

Total available feeding stuffs (in 1,000 tons)

Source of feeding stuffs	Available quantity	Calculated nutrients			Total digestible nutrients
		Digestible crude protein	Digestible carbohydrate	Digestible fat	
Dry fodder	1,11,000	1,010	34,580	883	36,480
Green fodder	1,00,000	1,090	10,090	250	11,562
Concentrates	1,595	153	975	150	1,168
Cotton seed	2,300	300	690	367	1,318

If we calculate the amount of nutrients available per head of cattle (Table II) and compare it with the normal food requirements of an adult animal weighing 500 lb., leaving aside the growth and production allowances, it will be recognized that, even under normal conditions, the supply of food falls short of their requirements.

TABLE II

Foodstuffs available per head of cattle

	Available in India in tons	Available per animal per day for 216 million heads of cattle in lb.	Normal requirement for an animal of 500 lb. body weight in lb.
Total digestible nutrients	5,10,13,000	1,456	3.9
Total digestible crude protein	27,00,000	0.970	0.3
Dry matter { Dry fodder	11,10,00,000	3.17	10.0 to 11.0
Green fodder	10,00,00,000	0.55	
Concentrates	38,00,00,000	0.11	

It has, however, been pointed out by Wright [1937] that the total available nutrients may be sufficient to meet the requirements of milk production alone, assuming for the moment that the requirements for the growing stock and for working cattle will be met from grazing, tree fodders and from other miscellaneous sources. Wright also observes that underfeeding is apparent from the slow rate of growth, the late maturity and the long dry periods. It is quite usual for a cow to drop her first calf at four to five years of age and to have calving intervals up to 600 days. That these are not hereditary defects is proved by early maturity and controlled breeding experiments in herds where the feeding and management are satisfactory.

The above observations, therefore, suggest that, even under conditions where rainfall is within normal range, the feeds available for livestock from all sources so far known are just on the border-line of sufficiency. Scarcity, however, is immediately felt if the monsoon fails. During the last century there were about ten drought periods, some of great severity. The recent famine (1937-40) which affected Sind, Rajputana and the south-western Punjab is said to have been the severest

within living memory, and to have caused very heavy mortality from disease as well as from starvation and thirst [Chandra, 1939].

Moreover, the shortage of water resulted in a complete failure of fodder and crops, so that most of the good cattle of the locality had to be sent away to other places, sold at such ridiculously low prices as 8 annas per cow (personal communications). [Chandra 1939 A, and B] reported the death of a large number of cattle *en route* to the grazing areas of the United Provinces, due to cold or to the inferior and unsuitable grazing in the jungles. It was reported that about four lakhs of cattle died in Karachi district alone. According to information supplied by the Marketing Officer, Sind (personal communication) there were approximately 5,61,000 cattle before the famine in the Thar Division of the Tharparkar district. By May 1940, when the famine was still raging, about 2,69,000 of these had died, 1,17,000 were exported and 10,000 sold. In the affected areas of the Rohtak district, the cattle population decreased by 30 to 60 per cent. Sheep and camels were comparatively in a happier position, as they can subsist on the flora available in the xerophytic areas (Plate VI, figs. 1 and 2). The effect of this famine on the livestock in the Hissar district is shown in Table III, where the census figures of 1935 are compared with those of 1940.

The scarcity of draught animals was so acute that the poor cultivator, whose bullocks had died of starvation, had to yoke his wife and children to the plough.

The remaining animals also manifested deficiency diseases, especially these due to lack of vitamin A, such as night blindness, xerophthalmia, abortion, retained placenta and calf scours. Most of the survivors which were hidebound developed depraved appetite. Besides, the calf and lamb crop, the carrying capacity and the milk production were reduced.

The question then of keeping these animals alive during these lean periods is one of vital interest to animal nutrition workers whose duty it is to find new sources of fodder, which may be exploited for this purpose.

EXPERIMENTAL

Methods and material

During a visit to the famine-stricken areas it occurred to the writer that it might be possible in an emergency to utilize factory or agricultural by-products or even weeds found either around these areas or within easy reach as rations for livestock. Other suggested famine fodders were xerophytic shrubs such as *Zyziphus nummularia*, W. and A. (shrub berry), *Lamanea muticaculis* Hk. f. (ghobhi grass), *Tamarix articulata* Vahl (loi), *Alhagi camelorum* Fisch. (bandrari), *Capparis aphylla* Roth,

TABLE III
Livestock census of the Hissar, Rohtak and Gurgaon districts in 1940 as compared with that of 1935

Year	Bulls		Bullocks	Cows	Calves		Buffalo		Calves		Sheep	Goat	Horses & Ponies		Mules	Donkeys	Camels
	Hissar	Others			Male	Female	Bulls	Cows	Male	Female			Male	Female			
	HISSAR																
1935	1216	191	101914	121397	69026	86915	954	90854	30934	64180	161725	206483	1283	3610	105	17204	42142
1940	1004	40	58137	67228	23701	45197	703	46879	13149	30927	254075	109476	819	2075	73	11013	20550
ROHTAK																	
1935	1249	...	127726	87459	124224	124224	1222	92336	97140	50384	84721	1701	2419 557 (ponies)	190	18170	3309	
1940	1102	...	92396	69511	100211	755	60043	67444	50437	89184	902	1278 335 (ponies)	147	12382	4175		
GURGAON																	
1935	901	959	120001	93866	58377	57752	2322	97107	27240	61370	45855	130970	5090	5737	1024	...	4517
1940	572	855	109295	84076	42474	47198	1804	77777	78429	54895	80410	151184	2341	4201	120	...	3954

NOTE:—The above figures are taken from *Famine Bulletin*, No. 16, 1940, P. 4, by courtesy of Sir Colin C. Garbet, Financial Commissioner, Government of the Punjab

TABLE IV

	Percentage on dry matter basis							CaO	P.O.
	Dry matter	Total ash	Crude protein	Ether extract	Fibre	N. F. extract	Soluble ash		
Mung (<i>saccharum munga</i> Roxb.)	94.47	3.703	4.325	1.681	45.60	45.291	2.811	0.316	0.236
Bajra (<i>perisetum typhoidum</i>)	94.20	7.350	3.631	0.610	36.64	51.739	3.115	0.086	0.160
Corn (<i>zea mays</i>) heart	95.28	3.507	3.681	0.835	35.07	57.487	1.621	0.086	0.265
Groundnut (<i>arachis hypogaea</i>)	96.48	4.482	7.713	1.641	65.86	20.304	2.482	0.427	0.278
Rice (<i>oryza sativa</i>) hulls	92.90	26.310	4.488	3.500	38.51	33.192	2.670	0.094	0.734
Cotton (<i>gossypium</i>) hulls	92.02	2.84	6.13	2.69	40.94	39.42
Wheat <i>blusa</i>	93.74	14.07	2.89	1.02	39.55	42.97	3.13	0.42	0.51

(*delia*), *Cassia lanceolata* Wall. (*dhabeji*), *Farsesia hamiltoni* Royle. (*lotia* grass), *Prosopis pubescens* Benth. (*debi*), *Prosopis specigera* (khejari), *Salvadora persica* Linn. (*jar*), *Tribulus terrestris* Linn. (Bakra) *Yucca elata* (soap weed), *Yucca macrocarpa* (Spanish dagger), *Yucca glauca* (Bear grass), *Opuntia elatior* (prickly pear), *Opuntia* Spp. (spineless cactus), *Euphorbia nerifolia*, *Salsola foetida*, *Euchlaena mexicana*, Sotol, Old man's salt bush etc., and trees like *Zizyphus jujuba* (ber), *Dalbergia sisoo* (*shisham*), *Ficus religiosa* (*pipal*), *Melia azadirachta* Linn. (*neem*), *Acacia arabica* (*kikar*), *Grewia oppositifolia* (*pastawunah*), *Morus alba* (mulberry) *Bauhinia variegata* (*kuchnor*), *Adina cordifolia* (*haldu*), which are commonly found in arid and semi-arid areas.

Many of these are actually fed by the people to livestock during famine, a fact borne out by numerous trees lopped to stumps (Plate VI, fig. 3). Indeed little except poisonous or thorny plants is allowed to remain in the sun-scorched pasture areas (Plate VI, figs. 4 and 5). No systematic work has, however, been carried out on these little-known fodders. The present work was undertaken to find out systematically and scientifically some sources of readily available 'famine fodders' which, when fed over prolonged periods, would not produce a deleterious effect. In the course of these investigations, the possibilities of feeding *munj*, *beans*, groundnut husk, *bajra* husk, rice hulls and molasses have been considered. Their chemical composition as compared with that of wheat *blusa* is shown in Table IV.

The present article, the first of the series, deals with the possibility of using *munj* and molasses as feeds during periods of scarcity.

MUNJ AND MOLASSES

Munj (*Saccharum Munja* Roxb.). Since *munj* is available in very large quantities, attention was first directed to its possible utilization as a feed. This was done in spite of the fact that Bor [1941] considered it of little or no value as a fodder grass; moreover it is very rough with tough serrated leaves. *Munj* grows wild over millions of acres and, except that its young shoots are indifferently browsed upon by animals for variety and a fraction of its total produce used for thatching huts, most of it is disposed of by burning when mature.

This plant shows marked xerophyllous adaptations. Although it attains its maximum development in moist land, it thrives in typically xerophyllous localities as well [Blatter and McCann, 1935]. Bor [1941] pointed out that it could grow on the driest of soils, though in such places as the Rajputana desert its growth was stunted.

Molasses. As grain is available only to a fraction of the cattle population even in normal times,

the possibility of providing an adequate supply in times of total or partial crop failure is out of question. A search for a cheap source of carbohydrate suggested the possible use of molasses, which is the chief by-product of the cane sugar industry. According to Morrison [1937], Snell and Taggart [1933], cane molasses is palatable and is relished by stock. It contains 55 to 65 lb. of total digestible nutrients per 100 lb. which is about 70 per cent of what is supplied by corn grain.

With the rapid development of the sugar industry, the annual output of molasses by various factories is estimated to be well over 6,00,000 tons. Only a small fraction of this can be utilized and the disposal of the remainder is an embarrassing problem for factory owners. The fermentable nature of molasses on dilution and the nuisance that it causes when thrown into open pits require its immediate disposal.

Several samples of molasses obtained from different factories gave the following average values on analysis:

Dry matter	Total ash	Crude protein	CaO	P ₂ O ₅	S
76.10	8.27	1.74	0.97	0.07	0.79

It may be pointed out that in the process of sugar manufacture, the juice is clarified and purified with lime and sulphur dioxide. The residual syrup, after the grains of sugar have been separated by a centrifuge, contains, in addition to about 55 per cent of sugars, fairly large quantities of calcium oxide and sulphur, which require adjustment for long feeding trials.

FEEDING EXPERIMENTS

Feeding trials, which were made as comparable with field conditions as possible, were conducted on eight adult healthy hill bulls. The *munj* and molasses ration, which was poor in protein and high in calcium, was supplemented with mustard cake and wheat bran, and tried for (1) their palatability, and (2) their effect on the health of the animals. Before feeding the *munj* was chopped, beaten with sticks to remove the adhering dust and moistened with water a few hours before it was given to the animals. The extent of moistening was so adjusted that it did not prick when pressed between the hands. At this stage it contained about 40 per cent moisture. This moistened material was then placed in the manger, and mixed well with cake and bran which had been soaked previously in water. Molasses mixed in equal proportions with water was sprinkled over this mixture and the whole mass stirred up thoroughly. In addition to the above, salt licks *ad lib* were supplied and 1 to 2 lb. of green tree leaves were given twice a week to provide carotene, since avitaminosis A is one of the chief deficiencies

in famine conditions. The carotene content of some of the common trees is given in Table V.

TABLE V
Carotene content per kg. dry matter

	mg.	D ry ma ter (per cent)
<i>Jhar beri</i> leaves	195.3	25.60
<i>Shisham</i> leaves	155.1	23.85
<i>Neem</i> leaves	103.6	22.20
<i>Ber</i> leaves	256.4	20.6
<i>Pipal</i> leaves	80.8	26.7
<i>Babul</i> leaves	132.4	42.3
<i>Dub</i> grass	250.0	16.0
<i>Dub</i> grass roots	1.2	60.8

Further observations on the chemical composition of some of the tree leaves (Table VI), e.g.

shisham, *likar*, *ber*, *neem*, *pipal*, *pastawunah* mulberry, *kachnar* and *haldu*, indicated that in addition to being sources of carotene, they are moderately rich in protein and can, therefore if available in sufficient quantities in the famine areas, replace cake, thus reducing feeding costs.

The following adjusted ration was fed to animals weighing 500 lb.

Mung	.	.	9 lb.	} 1 lb. green leaves or grass or roots of grass twice a week.
Molasses	.	.	2 „	
Mustard cake	.	.	1 „	
Wheat bran	.	.	1 „	
Salt	.	.	ad lib.	

This ration provided approximately 0.376 lb. digestible crude protein and 3.46 lb. starch equivalent and thus compared favourably with the normal requirement.

TABLE VI
Percentage composition on dry basis

Tree leaves	Total ash	Crude protein	Fibre	N. F. extract	Crude fat	CaO	P ₂ O ₅
<i>Haldu (Adina cordifolia)</i> . .	7.93	15.26	12.26	60.19	3.93	2.41	0.26
<i>Kachnar (Bauhinia Variegata)</i> .	8.54	13.15	29.37	46.82	2.12	3.40	0.42
<i>Mulberry (Morus Alba)</i> . . .	3.80	13.99	15.71	49.70	6.80	2.74	0.45
<i>Pastawunah (Grewia Oppositifolia)</i>	14.90	16.37	16.68	43.77	8.38	5.00	5.85
	14.16	10.12	14.09	54.81	6.82	0.68	0.57
<i>Ber (Zizyphus Jufuba)</i> . . .	10.35	12.80	11.67	60.15	1.99	4.12	0.39
<i>Neem (Melia Azadirachta)</i> . .	11.67	15.31	13.78	58.47	3.21	4.85	0.47
<i>Pipal (Ficus Religiosa)</i> . . .	14.65	12.68	21.55	47.32	2.68	5.37	0.45
<i>Shisham (Delbergia sisoo)</i> . .	9.12	16.27	22.63	49.78	2.98	3.22	0.37
<i>Paker (Ficus Infectoria)</i> . . .	12.62	10.90	23.97	53.65	2.14	2.09	0.41

RESULTS AND DISCUSSION

(a) *Observations on adult animals*

In the first experiment observations were made on two healthy adult hill bulls, seven and eight. They were fed 50 per cent. of their normal ration

for 12 days, so that before they started on the experimental diet their condition approximated to that of those in the drought areas. During this period the animals lost 20 to 40 lb. of body weight (Fig. 1).

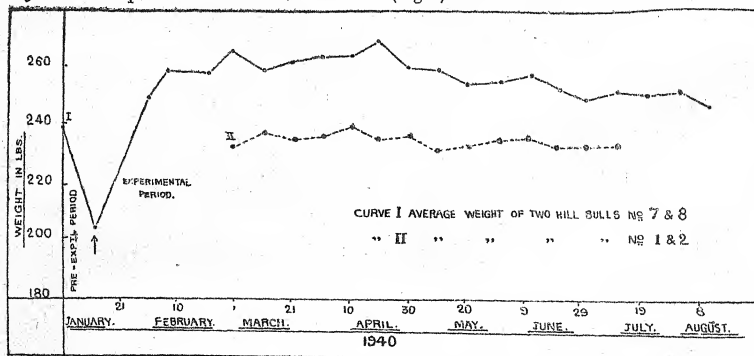


FIG. 1. Weight of adult bulls on *munj*-molasses ration

They were then fed the *munj*-molasses ration. All the animals relished the ration after acquiring a taste for it, and within 20 days of the commencement of this feed not only did they regain their lost weight but put on 15 to 20 lb. more than their original weight which had been at a stationary level on a normal scheduled ration. During the next 20 days the animals did not gain in weight at the same rate as in the first three weeks on account of a severe cold wave. To prevent its being eaten at night, the animals were not provided with bedding. However, as soon as the cold wave was over, the animals again started putting on weight till they reached a peak of about 30 lb. more than their original weight, or 65 lb. more than their weight before they started on the experimental ration. At this stage the animals looked healthy and their coats were soft. Two more hill bulls, one and two, were added to this group 52 days after the commencement of the experiment. In their case the period on half-feed was dispensed with. Though they did not like the new ration to begin with, they soon acquired a taste for it, ate all they were given and gained in weight.

In view of the fact that when famine conditions are improved at the onset of the rains, and the animals are given work, these animals were sent out to work on the Institute farm for two to four hours daily. They showed an initial decrease of 5 to 10 lb. in body weight, but later the body weight remained stationary.

After about 100 days of feeding on this dry roughage, the animals showed indications of night blindness. Consequently, 2 lb. per head of *shisham* or *pipal* or *ber* leaves were included in their ration once a week. The eyesight of the animals improved and there was no recurrence of this condition.

In order to study the effect of weather on the continuous feeding of such a crude roughage as *munj* and molasses, especially in view of the fact that there is some prejudice against the prolonged use of the latter in summer [Labh Singh and Ghambhir Singh 1935], this feeding experiment was continued from 1 January to 10 August 1940 covering the winter, summer and monsoon seasons. During the entire course of this period the animals showed no signs of ill-health. Sexual appetite, however, became prominent in May and June.

When these animals had been on this ration for about two months, a metabolism experiment was conducted to investigate the digestibility and nutritive value of *munj*. These observations were carried out on hill bulls one, seven and eight weighing 248, 264 and 264 lb. respectively at the beginning of the experiment. The experiment lasted 10 days during which period accurately weighed foodstuffs were fed and the residue left after 24 hours was deducted from the total amount offered to find the net quantity consumed. The faeces were collected daily for 10 days and weighed. At the end of this period samples of foodstuffs and faeces were analysed. The results are given in Table VII.

TABLE VII
Chemical composition (on dry basis)

	Wheat bran	<i>Munj</i>	Rape cake	Molasses	Faeces H. B. 1	Faeces H. B. 7	Faeces H. B. 8
Ash	0.423	6.838	7.861	13.070	12.860	12.090	12.630
Crude protein	10.694	2.916	33.025	2.723	5.316	5.189	5.162
Ether extract	2.731	1.533	16.440	..	1.770	1.770	1.928
Fibre	17.520	41.230	9.340	..	32.340	32.590	32.470
Nitrogen-free extract	59.632	47.483	33.334	84.207	47.714	48.361	47.810

It was observed that the protein digestion of *munj* is negative, and ether extract and total carbohydrates are digested to the extent of 16.31 and 30.69 per cent respectively. The digestible nutrients are given in Table VIII.

TABLE VIII
Digestible nutrients per 100 lb. of *munj*

Crude protein	Carbohy- drates	Ether extract	Total digest- ible nutrients	Nutritive ratio	Starch equiva- lent
.....	27.23	0.26	27.78	...	3.50

Since the digestibility values for molasses, wheat bran and rape cake used for calculating the digestible nutrients of *munj* are obtained from the observations of Morrison [1937], it is reasonable to consider these values as near approximations. It is, however, obvious from the data, as was also indicated by the chemical analysis, that *munj* is not a maintenance roughage and adequate provision of protein and carbohydrate is necessary.

From this feeding trial, which covered almost all the seasons of the year, it was observed that not only did the animals maintain good health all along but gained an average weight of about 40 lb. (Fig. 1) in spite of being given a certain amount of work.

(b) Observations on growing animals

When a ration is recommended for general use it is not in practice fed only to adult stock but to stock of all ages.

An experiment similar to that conducted upon hill bulls was, therefore, carried out on calves to study the effect of this poor roughage on growing animals. Eight Hariana bull calves of four to six months of age from the Institute dairy were divided into four groups of two each. The first group was fed on the basal ration consisting of *munj*, molasses, wheat bran and rape cake; the second on the basal ration plus $\frac{1}{2}$ lb. each of *pipal* and *ber* leaves; the third on the basal ration plus grass roots (as in the famine area where tree leaves are not available, grass roots are fed); and the fourth on a normal dairy ration.

The calves were divided into these groups on the basis of age and weight. The starting weight was taken as the average of four days' body weight.

The basal ration consisted of:

<i>Munj</i>	7 lb.
Molasses	3 "
Mustard cake	2 $\frac{1}{2}$ "
Wheat bran	2 "
Salt	ad lib.

The normal ration as fed to dairy calves consisted of:

Green fodder	20 lb.
Dry roughage	8 "
*Concentrates	4 "
Salt	1 oz.
Mineral mixture	1 "
	Per cent
*Wheat bran	40
Gram husk	20
Groundnut cake	20
Rape cake	10
Gram chuni	10

These calves were weighed daily before any feed was offered to them. They were given about a couple of hours' exercise, after which they were allowed to remain in the paddock for as long a period as weather permitted. The animals were muzzled when they went out of the stalls.

Feeding trials started on 1 September 1940. They were offered a reduced ration for nine days and then fed on the experimental ration. For the first ten days the animals did not apparently relish the ration as is evident from (Fig. 2) indicating

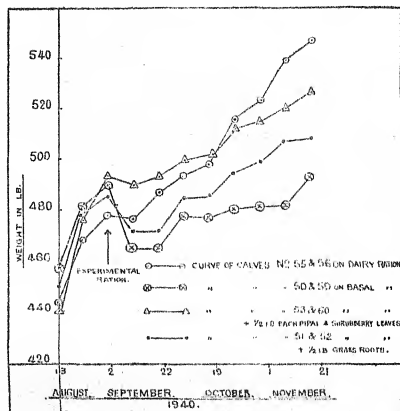


FIG. 2. Growth of calves on *munj*-molasses ration

loss in weight. They left 3 to 5 lb. residue. These animals took a little longer than the adult cattle to take to this diet, as was to be expected because prior to being put on this ration they were fed a rich and palatable ration which included succulent roughage and grazing. Nevertheless, the control group did not show any increase in weight for the first few days, probably because of their new surroundings and the absence of grazing.

Munj proved most palatable to the animals when chopped, beaten to remove dust and moistened for about 24 hours. On moistening 7 lb. of dry *munj* would weigh about 11 lb. Molasses was mixed with rape cake soaked overnight in water in winter or about four hours in summer. This concentrate was thoroughly mixed with the roughage, and wheat bran was spread on the surface and lightly mixed.

The feeding observations were carried out for 90 days from the 1 September to 29 November 1940. The relative gain in weight by different groups of calves is given in Table IX and the rate of growth is indicated in Fig. 2.

It will be observed that by the end of 81 days under trial, groups I, II and III gained in weight respectively 50.9, 70.03 and 59.4 per cent of the

weight gained by the control group. The animals in the first three groups invariably left a residue of 1 to 3 lb. during the course of this observation

which suggests that the ration could be slightly decreased. All through this period the animals maintained good health.

TABLE IX

Group	No. of calf	Date of birth	Live-weight at the beginning of the experiment	Average	Live-weight at the conclusion of the experiment	Average	Total gain in weight of the group	Per cent gain in weight	Gain in weight expressed as per cent of control group
I Basal ration	50 59	10-2-39 26-4-39	528 401	464	566 434	500	36	7.9	30.9
II Basal ration $\frac{1}{2}$ lb. <i>pipal</i> , and $\frac{1}{2}$ lb. shrubbery leaves	53 60	7-3-39 26-5-39	523 451	487	582 498	540	53	10.9	70.03
III Basal ration Grass roots and grass 1 lb.	51 52	18-2-39 28-2-39	455 490	472	514 518	516	44	9.2	59.4
IV Control group on dairy ration	55 56	12-3-39 14-4-39	490 472	482	562 552	557	75	15.5	...

GENERAL DISCUSSION

From the foregoing observations, it appears that in spite of the long-term feeding experiments, the two general requirements, i.e. (a) palatability and (b) maintenance of health, were satisfied. It may, however, be pointed out that *munj* is very tough, and when the animals are first changed over to this ration from the normal ration they refuse it. Thus in practice when *munj* roughage is to be used, the animals should either be gradually introduced to it or they should be given a reduced allowance for about a week. In the famine areas, however, conditions are obviously different, for cattle will even feed greedily upon the bone-dry *munj* of roof thatchings. When these animals were offered the *munj*-molasses mixture they relished it immensely.

There appears to be some prejudice against molasses as cattle feed. It is believed by cultivators and cattle breeders that molasses produces too much heat. Labh Singh and Ghambir Singh [1934] in their experiments on feeding molasses observed that in winter months (January to April) molasses could be fed economically up to a maximum of 4 lb. per animal (1,000 to 1,200 lb. body weight) per day without any ill effects, but in summer months (July to September) the animals deteriorated in health and their dung became watery and dark in colour. Some went off their feed, while in others the respiration became very rapid during the hotter part of the day.

In the temperate regions, however, Morrison [1937] and most of the other workers found it suitable for use all through the year. Not only has it been advocated as a rich source of carbohydrate [Henke 1934; Snell 1935; Skinner and King, 1936-37; Snell and Taggart, 1933; and Morrison, 1937], but of other essential food constituents as well. Harris, Mosher and Bunker [1933] found that molasses was second only to liver as a source of available iron. The total iron content of three samples varied from 3.2 to 11.3 mg. per 100 gm. and the availability ranged from 54 to 97 per cent. Cunningham [1934] observed that molasses is a valuable source of minerals, in particular of Ca and Mg.

Briggs and Hellen [1940], however, pointed out after a series of digestibility trials that the inclusion of large amounts of molasses lowered the digestibility of the nutrients in the ration, especially that of fat and protein.

In order to determine whether the cumulative^e feeding of molasses exercised any ill effects on milk production and breeding efficiency of cows, Henk [1934] fed it to cows for a period of seven consecutive years and found that throughout the long-term test there was no decrease or increase in milk production, though there was a slight increase in fat production as compared with that of the control group. There was no increase in the number

of abortions or any significant decrease in reproductive efficiency as a result of feeding molasses. In a fifteen weeks' experiment, however, he showed that cows fed on molasses averaged 1 lb. heavier per head than those on the control ration.

In the present observations from January till 8 August, it was observed that the dried tough *munj* which is ordinarily considered refuse, was relished when molasses was added to it. In fact, molasses acted as a condiment.

Health of the animals. Labh Singh and Ghambir Singh [1934] pointed out that bullocks receiving molasses in winter apparently enjoyed as good health as other animals, but, if the feeding was continued in summer also [*ibid* 1935], the animals went off feed and their faeces became dark and watery. Morrison [1937] stated that molasses was apt to produce scour in calves if they were allowed all the molasses they could eat. Galloway [1940], however, in Louisiana trials found that, if calves were fed small quantities in the beginning and the amount was gradually increased, there was no trouble from scouring.

In the present experimental observation on the Kumauni hill bulls who were fed the *munj*-molasses ration for about eight months and on the eight calves similarly fed for three months, it was noticed that all the animals were healthy and the bulls were capable of mild work.

ECONOMIC CONSIDERATIONS

During famine when home-grown feeds are rare the prices of purchased feeds are exorbitantly high, while money for buying feeds is not plentiful with the agriculturists who have suffered a total or partial crop failure, it becomes imperative to furnish the most in feeding value for every rupee spent.

The cost of the suggested *munj*-molasses ration will, therefore, be that of cutting and transporting *munj* plus the cost of transporting molasses, since the value of *munj* in rural areas and of molasses at the factory head will be negligible.

It is estimated that the cost of this experimental ration, suited to the needs of scarcity areas, is 25 to 35 per cent that of the standard ration.

SUMMARY

In this article, a reference is made to the famine problem in India. It has been pointed out that non-irrigated arid and semi-arid areas are occasionally subject to famine.

To help starving livestock to survive extreme drought conditions, efforts have been made to discover new sources of fodder.

The observations reported here were made on *urunj* (*saccharum munj* Roxb) as roughage and on molasses as the chief source of carbohydrate. This plant grows abundantly and thrives in typically xerophyllous places.

The first experiment was conducted on Kumauni bulls for about eight months. The animals gained in weight and remained in good health. In the second experiment Hariana calves were used. The calves also showed a fairly satisfactory growth as compared with the control group fed on the Institute scheduled ration. No animal showed any untoward symptom during the observation period of 90 days.

The cost of feeding both the Kumauni bulls and Hariana calves on the experimental ration was 25 to 35 per cent that of the standard ration.

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The author is indebted to Dr F. C. Minnett not only for valuable suggestions and advice but also for the encouragement he has given in elaborating the programme of work which forms the subject of this first article. He also wishes to express his gratitude to Dr K. C. Sen, Officer-in-charge, Animal Nutrition Section, for his valuable suggestions, Sir Colin C. Garbett, formerly Financial Commissioner, Government of the Punjab, for granting facilities for investigating the famine problem in the affected areas of the Punjab, the Directors of Veterinary Services, Punjab and Sind, the Revenue Minister, Jaipur State, his colleagues of this Institute, and the Staff of the Civil Veterinary Departments of the Punjab and Sind who have helped him in this work in the field and in the laboratory.

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USE OF COLOUR FRINGES IN BUTYROREFRACTOMETER FOR DETECTING ADULTERATION IN GHEE

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GODBOLE [1936] and Godbole and Sadgopal [1939] have advocated the desirability of noting the colour of the fringes visible when fat samples are examined, along with the refractometer reading. According to these authors, ghee of good quality gives invariably colourless fringes and is at times violet tinged. Hawley [1936] in reviewing some of the methods proposed for the detection of adulteration in ghee states that it is doubtful whether the colour of the fringes can be used as a reliable method, especially when it is possible to prepare artificial mixtures of fats having the same refraction.

Athavale and Jatkar [1938] extended these studies and recommended that measurement of both refractive index and dispersion on a Pulfrich Refractometer using the green and violet lines of mercury arc, provide a much more sensitive test. These authors, however, do not give any data as to the amount of various adulterants detectable by this method, the effect of feed on the fat secreted, etc. One of the main reasons why the problem of detection of adulteration in ghee still remains unsolved is the great variability in the composition of ghee, due both to the large number of breeds both of the buffalo and cow distributed all over India and the great variations in the foods fed in different places. Any method to be fool-proof must stand these tests. Neither the studies of Godbole and Sadgopal nor those of Athavale and Jatkar throw any light on these points. The present study was therefore undertaken to test in great details the utility of the Refractometer colour fringes in detecting adulteration of ghee.

EXPERIMENTAL

The apparatus used in the present studies was the Butyrorefractometer made by Carl Ziess. The source of light was diffused sunlight. All the readings were taken at 40°C. in duplicate, observing the usual precautions.

In all about 150 samples of ghee, known to be genuine, were examined. They represented samples collected from almost all the important parts of India, thus giving a very representative data. For the sake of convenience, figures of cow, buffalo, mixed and yellow* ghee are given separately in Tables I to IV. Almost all cow, buffalo and *yellow ghee samples were collected in co-operation with the Marketing

Agricultural and Veterinary officers of different provinces and states. Hence their purity is guaranteed. Most of the mixed ghee samples were obtained from ghee packers. Their origin, either, as pure cow or buffalo, is not known. So they have been classified as mixed.

TABLE I
Refractive indices and colour fringes of cow ghee samples

Sample No.	Place of origin	Refractive index at 40° C.	Colour of the fringe
1	Cuttack . . .	44.0	Violet
2	Dohad . . .	43.4	Pink
3	Asignabad . . .	43.6	Pink
4	Gulbarga . . .	43.5	Pink
5	Indore . . .	44.3	Violet
6	Anand . . .	43.7	Pink
7	Dargal . . .	43.7	Pink
8	Warangal . . .	43.9	Pink
9	Parachinar . . .	42.0	Orange
10	Gwalior . . .	43.8	Pink
11	Trichur . . .	44.0	Pink
12	Nalgonda . . .	41.8	Pink
13	Salon . . .	42.8	Diffused yellow/pink
14	Srinagar . . .	43.5	Pink
15	Kumbakonam . . .	43.0	Pink
16	Madura . . .	42.7	Pink
17	Calicut . . .	41.8	Pink
18	Ahmedabad . . .	41.8	Pink
19	Tanjore . . .	44.0	Pinkish violet
20	Coimbatore . . .	41.0	Diffused yellow/pink
21	Chittor . . .	44.3	Pinkish violet
22	Mohmedabad . . .	43.4	Pink
23	Tanjore . . .	45.2	Violet
24	Cuddapet . . .	41.5	Red
25	Cuddapet . . .	41.8	Red
26	Calicut . . .	43.2	Violet
27	Vizagapatnam . . .	44.1	Pinkish violet
28	Madura . . .	43.2	Pinkish violet
29	Coimbatore . . .	43.8	Pink
30	Bikkanoji . . .	43.2	Pink
31	Tenali . . .	43.9	Pink
32	Bezwada . . .	43.6	Pinkish violet
33	Shiyali . . .	44.6	Pinkish violet

* Yellow ghee is so called because of its yellow colour. Usually it is a mixture of cow, goat and sheep's ghee.

TABLE II

Refractive indices and colour fringes of buffalo ghee samples

Sample No.	Place of origin	Refractive Index at 40° C.	Colour of the fringe
1	Cuttack . . .	44.1	Violet
2	Dohan . . .	45.0	Violet
3	Aslagabad . . .	45.0	Violet
4	Gulbarga . . .	42.9	Pink
5	Kakra . . .	42.8	Pink
6	Farbhandi . . .	43.7	Pink
7	Indore . . .	44.3	Pinkish violet
8	Anand . . .	42.5	Orange
9	Dargai . . .	41.9	Orange
10	Warangal . . .	42.8	Pink
11	Parachinar . . .	39.0	Orange
12	Gwallor . . .	44.0	Violet
13	Trichur . . .	41.7	Red
14	Tricy . . .	43.4	Pink
15	Nalgonda . . .	42.5	Pink
16	Bider . . .	42.8	Pink
17	Salem . . .	43.7	Red
18	Srinagar . . .	44.0	Violet
19	Madura . . .	41.4	Pink
20	Calicut . . .	42.5	Pink
21	Dholka . . .	42.2	Pink
22	Proddatur . . .	43.7	Pinkish violet
23	Tanjore . . .	43.6	Pinkish violet
24	Coimbatore . . .	43.0	Pink
25	Chittor . . .	43.4	Pink
26	Tanjore . . .	42.5	Pink
27	Nizamabad . . .	42.6	Pink
28	Jalagao . . .	42.4	Pink
29	Tanuku . . .	43.5	Pinkish violet
30	Kumbakonam . . .	42.4	Pink
31	Calicut . . .	44.5	Pink
32	Annur . . .	42.4	Red
33	Kurnool . . .	43.1	Pink
34	Vizagapatam . . .	42.1	Pink
35	Madura . . .	44.1	Pink
36	Tinnavelli . . .	44.1	Pinkish violet
37	Coimbatore . . .	42.1	Pink
38	Bilkanoli . . .	44.2	Pink
39	Tenali . . .	43.5	Pink
40	Erode . . .	44.9	Pink
41	Bezawada . . .	43.4	Pink
42	Shiyali . . .	44.8	Pinkish violet
43	Banda . . .	40.2	Orange

TABLE III

Refractive indices and colour fringes of mixed ghee

Sample No.	Place of origin	Refractive Index at 40° C.	Colour of the fringe
1	Hosur . . .	44.1	Diffused yellow/pink
2	Ilhugoli . . .	44.2	Pinkish violet
3	Porbandar . . .	45.5	Violet
4	Lyallpur . . .	42.1	Pink
5	Kosimdra . . .	45.7	Violet
6	Dacca . . .	43.4	Diffused yellow/pink
7	Cuttack . . .	44.4	Violet
8	Lyallpur . . .	42.2	Pink
9	Gashati . . .	44.3	Violet
10	Kirkee . . .	42.5	Pink
11	Alahabad . . .	42.5	Pink
12	New Delhi . . .	42.5	Pink
13	Dhaka (Akola) . . .	43.1	Pink
14	Khadi (Akola) . . .	44.2	Violet
15	Kanlari . . .	44.4	Violet
16	Calcutta . . .	43.4	Pink
17	Kathal . . .	44.2	Violet
18	Hoshangabad . . .	42.9	Pink
19	Shillong . . .	46.2	Violet
20	Dhinoj . . .	43.0	Pink
21	Ballad . . .	43.9	Pinkish violet
22	Morenae . . .	42.6	Pink
23	Bombay . . .	42.2	Pink
24	Bombay . . .	42.7	Pink
25	Jubbulpore . . .	41.8	Pink
26	Jodhpur . . .	42.5	Pinkish violet
27	Jodhpur . . .	43.4	Pink
28	Jodhpur . . .	43.2	Pink
29	Ajmer . . .	43.7	Pinkish violet
30	Deogarh . . .	43.7	Pinkish violet
31	Darbhangha . . .	42.7	Pink
32	Darbhangha . . .	43.0	Pink
33	Khagaria-Moghyr . . .	41.8	Pink
34	Barnmer . . .	43.2	Pink
35	Chota Barnmer . . .	42.4	Pink
36	Barnmer . . .	41.8	Red
37	Patlala . . .	43.3	Pink
38	Patlala . . .	43.3	Pink
39	Calcutta . . .	43.0	Pink
40	Calcutta . . .	43.0	Pink
41	Calcutta . . .	42.6	Pink
42	Calcutta . . .	43.0	Pink
43	Calcutta . . .	42.6	Pink
44	Jodhpur . . .	43.7	Pinkish violet
45	Kharagron . . .	40.3	Orange
46	Pandharpur . . .	43.2	Pink
47	Medlar . . .	43.6	Pink changing to violet
48	Dohad . . .	45.3	Violet
49	Jhand . . .	40.5	Red
50	Banda . . .	41.5	Red

TABLE IV

Refractive indices and colour fringes of yellow ghee samples

Sample No.	Refractive Index at 40° C.	Colour of the fringe
1	42.8	Pink
2	43.4	Pink
3	42.4	Pink
4	43.2	Pink
5	43.4	Pink
6	42.0	Pink
7	42.6	Pink
8	42.6	Pink
9	42.6	Pink
10	43.1	Pink
11	42.3	Pink
12	42.7	Pink
13	41.7	Orange
14	41.7	Orange
15	41.5	Orange
16	43.0	Pink
17	42.4	Red
18	41.8	Red
19	41.7	Red
20	41.4	Red

DISCUSSION

Refractive indices. Looking at the figure for cow ghee samples given in Table I it will be seen that the refractive indices show a variation from 41.0 to 45.2. The corresponding ranges for buffalo, mixed and yellow ghee are 39.0 to 45.0, 40.3 to 46.2 and 41.4 to 43.4 respectively. These data therefore indicate that figures for refractive index range very widely from 39.0 to 45.2. The majority of samples, however, fall within a narrow range of 41 to 45. Even this range of variation is quite considerable to make this value of any use in checking adulteration in ghee. Figures for yellow ghee fall within the range for cow and buffalo.

Colour fringes for ghee.—Figures in the various tables show that genuine ghee shows either orange, red, pink or violet colour. To a certain extent this colour of the fringes is correlated with the figures for refractive indices. Thus fats having a refractive index of about 40 to 41 show orange or red colour. As the figure increases the colour changes to pink and further to violet. A large majority of samples show pink colour. Like all other chemical tests, therefore, the colour fringes also do not give a constant indications.

The Effect of temperature on the colour fringes.—The index of refraction of a fact and the colour of the fringe are closely interrelated and this is proved by the fact that if the refractive index is changed, e.g., by raising or lowering the temperature, there is a parallel change in the colour observed. In the Table V are given a typical series of results establishing this fact. The colour of the fringe changes from violet to orange as the refractive index shifts from 40.7 to 36.6.

TABLE V

Effect of temperature on the refractive index and colour fringes of ghee

Temperature °C.	25°	30°	35°	40°	45°	50°
Colour of the fringes.	Violet	Pink	Red (+)	Red (+ +)	Orange	Orange
Refractive index	40.7	40.8	43.8	41.0	37.6	36.6

The data show that the colour of the fringe cannot be taken as a constant characteristic of ghee or any other fat, and is determined by its refractive index.

Colour fringe of vanaspati.—In the course of the present studies, almost all the different brands of vanaspati, as well as edible oils, were examined to see how their colour fringes varied.

It would be rather out of place to give the detailed results here, but the net result of this study was that it was found that most of the samples gave a blue band. Their refractive index generally was higher than the range normally found for ghee. Vanaspati made from coconut oil gave a very low value for the refractive index and the colour of the fringe was red. By suitable blending of two vanaspaties or vanaspati with coconut oil, etc., it was quite possible to prepare a mixture having refractive index and colour fringe similar to that found for ghee. This is illustrated by some examples given in Table VI. For obvious reasons it has not been possible to disclose the names of vanaspaties used in these experiments.

TABLE VI

Refractive indices and colour fringes of mixture of different fats

Description of the mixture	Refractive index at 40° C.	Colour of the fringe
I. Mixture of vanaspati brands A & B :		
Brand A	34.6	Yellow/red
" " 5% + Brand B	50.5	Inky blue
" " 10% + " "	49.6	Inky blue
" " 20% + " "	48.8	Inky blue
" " 30% + " "	47.0	Violet
" " 25% + " "	48.0	Violet
" " 30% + " "	45.5	Violet
" " 40% + " "	43.5	Pink
" " 50% + " "	41.6	Red
II. Mixtures of vanaspati brands A & C		
Brand C	51.6	Inky blue
" " 65% + Brand C	45.6	Violet
" " 60% + " "	45.0	Violet
" " 55% + " "	44.6	Violet
" " 58% + " "	44.4	Pinkish violet
" " 57% + " "	44.1	Pinkish violet
" " 56% + " "	44.0	Pinkish violet
" " 55% + " "	43.4	Pink
" " 50% + " "	43.0	Pink
III. Mixture of coconut oil and groundnut oil.		
Coconut oil	35.5	Yellow/red
Groundnut oil	55.6	Light blue/Inky blue
Coconut oil 60% + Groundnut oil 40%	47.3	Inky blue
" " 55% + " " 45%	46.3	Inky blue
" " 50% + " " 50%	44.7	Violet
" " 45% + " " 55%	44.0	Pinkish violet
" " 40% + " " 60%	42.8	Pink
IV. Mixture of Coconut oil and Pig Depot fat		
Pig Depot fat	54.0	Light blue/Inky blue
Coconut oil 60% + Pig Depot fat 40%	46.6	Inky blue
" " 55% + " " 45%	45.3	Violet
" " 50% + " " 50%	45.0	Violet
" " 45% + " " 55%	43.2	Pinkish violet
" " 40% + " " 60%	41.8	Pink

This conclusively proves that there is no justification for taking the colour fringe of ghee as characteristic of ghee. This test has the same utility and limitations in its application as the refractive index taken by itself.

Refractive indices and colour fringes of ghee from animals fed on oil cakes

It is well known that the nature of the fat included in the ration has a great influence on the chemical composition of the milk secreted fat [Hilditch and Thompson, 1936]. The wide variation observed in fat from different species of animals, collected in different seasons and from different parts of the country, can in the main be ascribed to this cause. In the present studies some experiments have been carried out on the effect of feeding varying amounts of same cake to cows and buffaloes. The amount of cake included in the diet varied from 2 lb. to 10 lb. per animal per day. Each feeding trial covered a period of two weeks.

A typical series of data are given in Table VII below.

TABLE VII

Refractive indices and colour fringes of butter fat secreted by animals fed on sesame cake

Sample No.	Quantity of Sesame cake fed in lb.	Cow butterfat		Buffalo butterfat	
		Refractive index at 40°C.	Colour of the fringe	Refractive index at 40°C.	Colour of the fringe
1	—	43.0	Pink	4.18	Red
2	2	43.2	Pink	43.7	Pink
3	4	44.4	Violet	42.8	Pink
4	6	45.5	Violet	44.5	Violet
5	8	46.8	Blue	45.0	Violet
6	10	46.4	Blue	45.0	Violet

These results once more emphasize the fact that the colour fringes change rapidly with the feed given to animals and hence cannot be taken as an inherent characteristic of butterfat.

Detection of adulteration using refractive indices and colour of the fringes

Some experiments on the detection of adulteration in ghee by noting the refractive index and colour of the fringes were carried out. It was found that unless the characteristics of the original ghee sample were known, it was ordinarily not possible to classify samples adulterated with 25 per cent of foreign fats even as suspicious.

SUMMARY

(i) Ghee samples from different parts of India show wide range in their refractive indices (39.0 to 45.2) and colour of the fringes. The nature of the fringe colour may be of some value in a small district where only one type of ghee is produced, but the information obtained regarding the quality of the ghee by this method cannot be any better than that given by the refractive index.

(ii) Owing to the great variation in the colour bands given by ghee from different parts of India, adulteration upto anything less than 25 per cent cannot even be suspected.

(iii) There is evidence to show that the colour of the fringe can be artificially manipulated, e.g. by varying the temperature at which the reading is taken or by adjusting the refractive index of a mixture of fats which need not contain ghee.

(iv) A close relationship exists between the colour of the fringes and the refractive indices.

(v) The colour of the fringe and the refractive index are affected by the nature of the food fed to milch animals.

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COMPARATIVE STUDY OF MILK AGAR AND MODIFIED MILK AGAR FOR THE BACTERIOLOGICAL EXAMINATION OF MILK

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 (Received for publication on 14 July 1943)

Milk agar recommended by the Ministry of Agriculture [1934] is generally used to determine the number of bacteria in milk by the plate

method. As the prices of the constituents of this medium, namely peptone and leuco, have greatly increased due to war, Barkworth and Davis [1942]

have modified the standard milk agar by decreasing the concentration of peptone and lemco from 0.5 and 0.3 per cent to 0.2 and 0.1 per cent respectively. These authors claim that their modified medium is as good as the standard milk agar, and the mean difference in counts obtained by the use of these two media is not significant. Hence they recommend the use of the modified medium as a suitable substitute for standard milk agar for the plate counts of milk.

Barkworth and Davis have examined raw (cow) and pasteurized milks with the modified medium. As any medium suggested should have a general applicability to all kinds of milk produced and handled under different conditions, the present investigation was undertaken with a view to testing the suitability of the modified milk agar when compared with the standard milk agar for the plate counts of different types of milk, such as

cow, buffalo, mixed (cow and buffalo), pasteurized and village produced milks. Altogether 308 samples of milk were examined for the purpose.

EXPERIMENTAL

The dilution technique of the Ministry of Agriculture [1934] adopted by Barkworth and Davis was used in the present study.

The logarithms of the plate counts obtained in each case by the use of the two media were taken into consideration, and the significance of the mean differences in logarithms between the two was determined by carrying out the *t* test as done by Barkworth and Davis.

The mean differences in logs of the plate counts obtained by the use of the two media, standard errors of the mean differences and the significance of the differences are given in the following Table.

Types of milk	Number of samples	Mean differences in logs of the plate counts	Standard error of the mean difference	Significance of the difference
Cow herd milk *(I.D.I. Farm)	79	-0.0405	0.0482	' <i>t</i> ' = 0.8408 N.S.†
Buffalo herd milk (I.D.I. Farm)	80	-0.0359	0.0434	' <i>t</i> ' = 0.7907 N.S.
Mixed milk (cow and buffalo) (I.D.I. Farm)	52	-0.0416	0.0380	' <i>t</i> ' = 1.0947 N.S.
Pasteurized milk (I.D.I. Farm)	45	-0.0430	0.0604	' <i>t</i> ' = 0.7119 N.S.
Village produced milk	52	-0.0263	0.0476	' <i>t</i> ' = 0.5525 N.S.

*I.D.I.—Imperial Dairy Institute

†N.S.—Not significant

DISCUSSION

Barkworth and Davis have stated that a medium could not be considered as a suitable substitute if the mean difference exceeded 0.1 log. The above results show that the mean differences in logs of the plate counts of the two media are less than 0.05 log in all the cases, and also they are not significant as shown by the *t* test. The results obtained by this investigation, therefore, support the findings of the authors of the modified milk agar. The modified medium can, therefore, be suitably used in the place of the standard milk agar for the routine bacteriological examination of the milk of the five types, referred to above, thus economizing in the use of material during the war exigency.

It has also been stated by Barkworth and Davis that the modified medium compares favourably with the standard milk agar from the point of view of mean count and colony size, but in the course of this investigation it was observed that the size of the colony was comparatively smaller on the former medium than on the latter.

SUMMARY AND CONCLUSIONS

1. The suitability of the modified milk agar recommended by Barkworth and Davis as compared with the standard milk agar for determining the number of bacteria in milk by the plate method has been studied.

2. The mean colony counts obtained on the modified milk agar with all the types of milk examined are not significantly different from those obtained on the standard milk agar.

3. The colony size on the modified medium is comparatively smaller than that on the standard milk agar.

4. The modified medium may be used in the place of the standard milk agar, even for the kinds of milk available in India for their routine bacteriological examination, thus economizing in the use of costly material especially in war time.

ACKNOWLEDGEMENTS

Thanks are due to Mr Zal R. Kothavalla, Director of Dairy Research, for his keen interest in the work.

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*THE SYSTEMATIC POSITION OF *ORNITHODORUS CROSSI* BRUMPT (1921)

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(Received for publication on 5 June 1943)

(With Plates VII and VIII)

THE name *Ornithodoros crossi* was proposed by Brumpt [1921] for a species of tick occurring in the Punjab. In 1930 Pavlovsky, working in Russia, produced evidence to show that the name was antedated by *O. papillipes* Birula. He also forwarded to Nuttall and Warburton in Cambridge for examination a consignment of Russian forms of *O. papillipes*, and these were found by them to be identical with *O. crossi* Brumpt. Brumpt himself later agreed that *O. crossi* Brumpt was synonymous with *O. papillipes* Birula.

The writer, during the course of his studies on the morphology of the Indian form of *O. papillipes*, found that it lacked certain characters which had been reported by Pavlovsky [1930] in *O. papillipes* and which had been utilized by him in distinguishing the latter from *O. choldkovskiyi* Pavlovsky (1930) and *O. tholozani* (Laboulhene and Megnin 1882). The most important of these characteristics is the tuft of denticulated hairs arising from the conical antero-ventral surface of the body in front of the camarostome. In the Indian form the male hypostome has nearly parallel sides, while the female hypostome shows some widening at the base. In the Russian form, the hypostome has parallel sides in both sexes. In the two forms there is also a marked difference in the disposition of the hypostomal teeth and in the size of the anus, vulva and basis capituli. The differences between the two forms are summarized in the table on the next page.

It will be seen from the table that the Indian form of *O. papillipes* differs in several fundamental characters from the Russian form as described by Pavlovsky [1930] and should be considered a separate species and that the name *O. crossi* should be regarded as valid. Unfortunately Brumpt's description [1921] of *O. crossi* is inadequate, and the species is therefore redescribed below.

Female. When fed, body dark gray slightly tinged with green, or slate colour. Unfed specimens, pale yellow. Form, more or less elliptical, the anterior end being a little drawn out, forming a beak-like protuberance. Engorged specimens measure 5.0×4.0 to 9.0×5.0 mm. (average 8.0×4.5 mm.). The arrangement of the dorsal discs is as in the Russian form. Corresponding

depressions occur in unfed ticks. The cuticle shows a reticulate structure with irregular protuberances interspersed, these being largest marginally; it appears wrinkled in the unfed specimens; the centre of each mesh of network-pattern appears (Plate VII, fig. 7) to be occupied by a single hair.

The arrangement of the furrows on the ventral surface is similar to that in the Russian form of *O. papillipes*. Eyes are absent. The anal ring measures 0.2 to 0.3 mm., each anal plate bearing nine to ten hairs. The vulva is 0.65 to 0.7 mm. wide. The capitulum lies in the camarostome, with its posterior and lateral margins protruding. The latter terminate where the slightly movable 'cheeks' begin. The denticulated hairs on the conical protrusion of the ventral body surface are absent. There is, however, a tuft of short denticulated hairs on the roof of the camarostome, visible only when the camarostome is raised. The basis capituli measures 0.5 mm. The hypostome differs in the sexes, being longer than it is broad in the male and spatulate with parallel sides. The crown of small teeth is followed by two rows of big teeth, the border row consisting of three teeth, the median one of two only. In the female it is longer than the base and hirsute, with a border row of three teeth and the median row of two only. In both the sexes, the hypostome has a slightly bulging end. Two long hairs arise at the sides of the hypostomal base, as in all *Argasidae*.

Plate VII, fig. 6 shows the lateral view of the palp, with its dense growth of fine, smooth hairs. The articles show different relative lengths, particularly article No. 1 which is longer than that in the Russian form. The structure of the cuticle resembles that of *O. tholozani* (Plate VII, fig. 7, and Plate VIII, fig. 2). Tarsus 1 has a wavy dorsal contour, the dorsal spur not being as pointed as in the Russian form (Plate VIII, fig. 8). The Haller's organ is elongate oval. T. 4 has practically no papillae on the ventral border, and the dorsal spur resembles that of the Russian form of *O. papillipes*. *Male*: size 4.5×2.5 to 5.5×3.0 mm. (average 5.0×2.8 mm.). The hypostome is shorter than in the female and has smaller teeth.

*Paper read under the title, '*Ornithodoros crossi* Brumpt [1921] as a valid species and not a synonym of *Ornithodoros papillipes* Birula (1893)', at the 26th session of the Indian Science Congress 1939. Revised and rewritten.

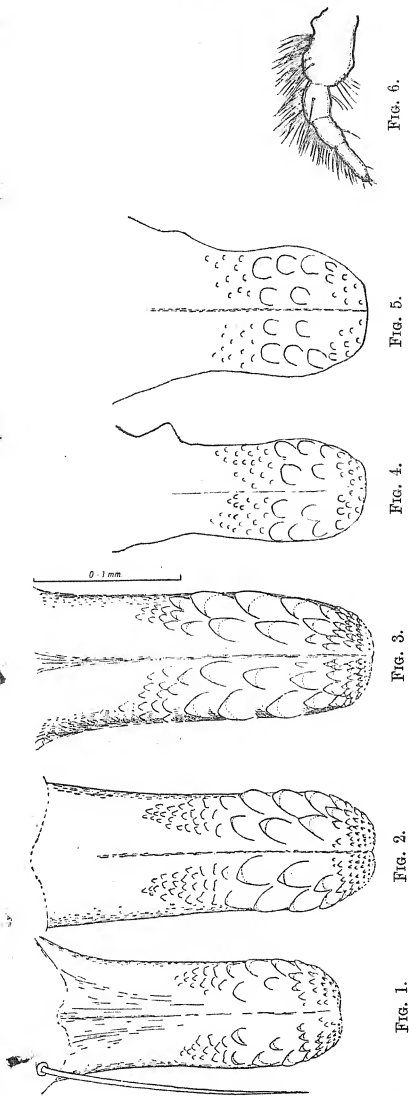


Fig. 6.

Fig. 5.

Fig. 4.

Fig. 3.

Fig. 2.

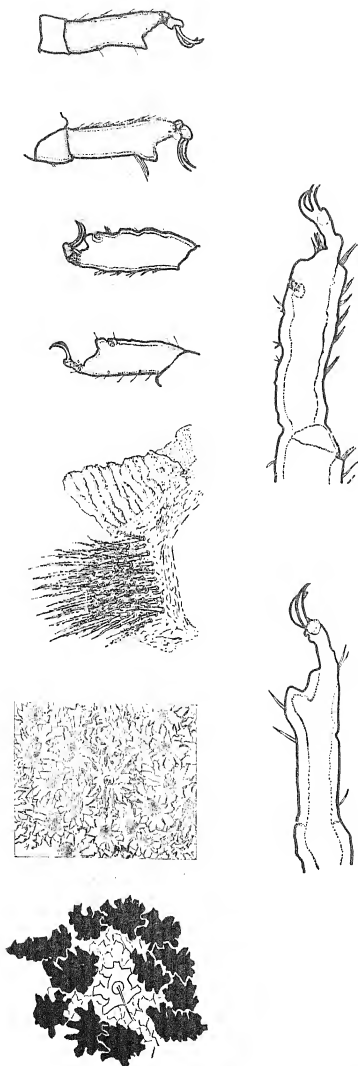
Fig. 1.

Fig. 4. Hypostome of *O. crossi* ♂
Fig. 5. Hypostome of *O. crossi* ♀
Fig. 6. Palp showing the dense growth of hairs of *O. crossi*.
Fig. 7. Structure of cuticle of *O. crossi*.



Fig. 7.

Fig. 1. Hypostome of *O. papillipes* ♀ (From Pavlovsky 1930).
Fig. 2. Hypostome of *O. papillipes* ♂ (From Pavlovsky 1930).
Fig. 3. Hypostome of *O. chotolkovskiy* ♀ (From Pavlovsky 1930).



- FIG. 1. Structure of cuticle of *O. papillipes*, the blackened areas denote plates in the network ; the white central plate is depressed and bears a hair (From Pavlovsky 1930).
- FIG. 2. Structure of cuticle of *O. tholozani* (From Brumpt 1936).
- FIG. 3. Denticulate hairs arising from the central antero-ventral surface of the body in front of the camerostome. (From Pavlovsky 1930).
- FIG. 4. Tarsus I of *O. chodolkooskyi*. (From Pavlovsky 1930).
- FIG. 5. Tarsus I of *O. papillipes*. (From Pavlovsky 1930).
- FIG. 6. Tarsus IV of *O. Chodolkooskyi*. (From Pavlovsky 1930).
- FIG. 7. Tarsus IV of *O. papillipes*. (From Pavlovsky 1930).
- FIG. 8. Tarsus I of *O. crossi*.
- FIG. 9. Tarsus IV of *O. crossi*.

It is assumed that the specimens, on which the foregoing description is based, are identical with the forms examined, and for which the name *O. crossi* was proposed by Brumpt in 1921. I have had occasion to examine several hundred specimens of the species of ticks in question from many localities in the Punjab and not one of these

conformed to the description and illustration of *O. papillipes* Birula, as given by Pavlovsky [1930]. In the circumstances there would appear to be good reasons for the assumption that the form of *Ornithodoros*, dealt with here and which I consider to be *O. crossi* Brumpt is conspecific with the type of original *O. crossi* Brumpt.

Characters	<i>O. tholozani</i> (From Nuttall and Warburton 1908 and from Brumpt 1936)	Russian form of <i>O. papillipes</i> (From Pavlovsky 1930)	<i>O. choldkovskyi</i> (From Pavlovsky 1930)	Indian form of <i>O. papillipes</i>
Colour	Not described	Grey or greyish yellow	Not described	Slate colour when fed and dirty yellow when freshly moulted
Size	Male 4 to 6 mm. long. Female 8 to 9 mm. long	4.2×2.5 mm. to 5.8×3.4 mm. 7.8×4.6 mm. to 8.2×4.6 mm.	Not described 7.4×3.7 to 4.2 mm.	4.5×2.5 mm. to 5.5×3.0 mm. (fed) 5.0×4.0 mm. to 9.0×5.0 mm. (fed)
Hypostome	In male spatulate, a crown of small teeth followed by two rows of three teeth, middle rows not far apart, then three to four rows of simple teeth. In female laeviculate, with two rows of three teeth median rows far apart	Both in male as well as in female about as long as broad, spatulate with parallel sides and two rows of three teeth on each side (Plate VII, figs. 1 and 2)	As in the Russian form of <i>O. papillipes</i> (Plate VII, fig. 3)	Male longer than broad, spatulate with parallel sides. A crown of small teeth followed by two rows, border row of three teeth while the median row of two followed by four to five rows of simple teeth (Plate VII, fig. 4) In female longer than the base. Lanceolate with border row of three teeth and median row of two only (Plate VII, fig. 5)
Palp	Not described	Short, thick and bearing many hairs.	The articles of the palp show different relative lengths and fewer hairs than in the Russian form of <i>O. papillipes</i>	Article No. 1 longer than in the Russian form. The palp bears a dense growth of hairs (Plate VII, fig. 6).
Structure of entole	Coarsely shagreened, one of every five or ten bears a long hair (Plate VIII, fig. 2)	Corporis derma reticulo rugosum (Plate VIII, fig. 1)	Not described	Same as in <i>O. tholozani</i> (Plate VII, fig. 7)
Denticulate hairs	Not described	Anterior conical protrusion of body bears 4 tuft of denticulate hairs (Plate VIII, fig. 3)	Anterior conical protrusion bears no such hairs	No denticulate hairs on the anterior conical protrusion of body but a tuft of short denticulate hairs placed on the roof of the Camerostome and only seen when the rostrum is raised.
Structure of tarsit 1 and 4	T. 4 with terminal dorsal protuberance prominent, pointed and directed distally, the terminal portion on tapering. Short hairs all articles, longest on tarsi.	T. 1 with wavy dorsal contour (Plate VIII, fig. 5). T. 4 as shown in plate VIII fig. 7, densely papillate on ventral border.	T. 1 less papillate (Plate VIII fig. 4). T. 4 with more pointed dorsal spur (Plate VIII, fig. 6)	T. 1 with wavy dorsal contour. Dorsal spur not as pointed as in the Russian form. (Plate VIII, fig. 8) T. 4 with hardly any papillae on the ventral border but with dorsal spur as in the Russian form (Plate VIII, fig. 9).
Size of the anal ring	Wider than long 0.3×0.35 mm. with 7 or 8 long hairs on each side	0.025×0.023 to 0.031×0.025 mm. each anal plate bearing 8 to 10 hairs.	Not described	0.2×0.3 mm. 8 to 10 long hairs on each side.
Size of the vulva	Not described	0.064×0.062 mm.	0.8 mm. wide	0.65 to 0.7 mm. wide.
Three small setae on one side situated near the base of the basis capituli	Not described	0.063×0.065 mm.	Not described	0.5 mm.
		Present	Not described	Not present

SUMMARY

Evidence is produced in this article to show that *Ornithodoros crossi* Brumpt [1921] is a valid species and not a synonym of *Ornithodoros papillipes* Birula [1895].

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A PRELIMINARY NOTE ON CUTANEOUS RINDERPEST

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(With Plate IX)

THE above is admittedly a coined title, and although only to some extent permissible, it explains more clearly than any other the subject-matter of this article.

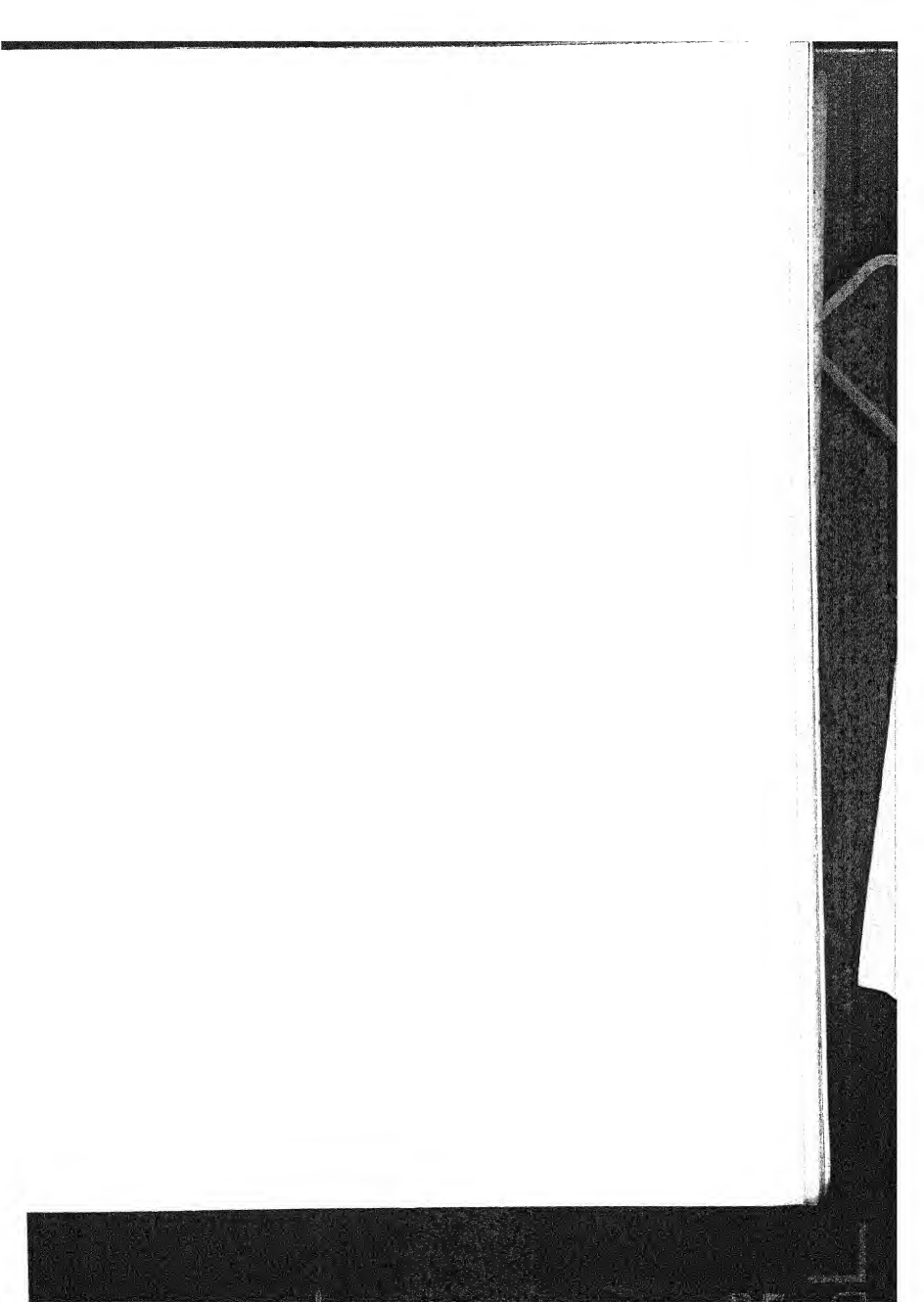
During a rather desultory study of the history of rinderpest in India from the mass of provincial and other records, a striking similarity appeared to prevail in the local names of this disease. The word 'rinderpest' for this disease (*rinder*—plural of *rind*—ox) is clearly foreign to India, giving little indication of symptoms or anything else beyond its being a pest affecting oxen. It seemed palpable that the terminology of this country, at least, was much more to the point and symbolic of the disease, for, in addition to meaning a disease of oxen, the word *mala*, in more or less universal use throughout India for the disease, means 'pox' from apparently the original religious conception that *mala*, being the mother-goddess of this disease, should be invoked by the use of her name for the good of the animal so afflicted. Other Indian names, such as *gau-bashanto* and *gubi* in Bengali, however, do not take on quite the same religious vncer, for *bashanto* means initially the Spring, although in process of time it has come to mean 'pox'—*gau* meaning cattle, hence cattle-pox in the spring time. This might be conceded to imply that a form of pox occurred mostly in the spring and was therefore, seasonal. *Gubi* literally means in Bengali a visible nodule—again most clearly a cutaneous eruption. To all intents and purposes, therefore, all the names which have adhered to this disease for thousands of years, point to the same conclusion, that rinderpest has been looked upon as a disease of the epidermis and not—as is today most definitely patent, a frequently fatal affection of the alimentary tract. And it is by no means beyond conjecture that the *patois* of countries outside Bengal, and India even, may prove, on further etymological research, that an equal dissimilarity exists between present-day conceptions of clinical rinderpest and the local names applied to it. For instance, in East Africa the local term is also reported to mean literally 'pox' and the same holds good for Java and the Dutch East Indies. However, without straining the point unduly, it seems reasonable to suggest that a period of some thousand or more years has changed the picture of rinderpest from a more or less benign cutaneous affection to the much more fatal alimentary syndrome met with today and

significantly without change or modification of its traditional names. On still further analysis of this surmise, there is presumptive evidence to suggest that in the wider spaces of the earth's surface a distinction even today exists between cutaneous forms of this disease and those confined symptomatically to the alimentary tract. Hutya and Marek [1926] record the incidence of two distinct types of rinderpest in Russia.

Russian authors attached much importance to the occurrence of this affection in cattle of the steppes and distinguished an exanthematous and non-exanthematous form of rinderpest; and also, in accordance with former medical opinion, regarded the appearance of the exanthem as a favourable sign in prognosis (the exanthem usually develops in protracted and therefore milder cases, as Croveri has recently proved in Africa). The cutaneous eruption is common in the course of some outbreaks, rare in others; thus Vrijburg never observed it in the West Indies, while Gartner in East Africa found it so common that the natives call the disease 'pox' (*djedri*). It also occurs in highly bred cattle independently of the course of the disease.

It might quite reasonably be suggested that the retention of the above note in modern literature has been the result of neglect on the part of more recent authors to delete an obsolete and unimportant reference, hence its survival; but it will have to be conceded that on a more intimate investigation of cause and effect the repeated report of the existence of two distinct forms of rinderpest in Russia—as described above is in keeping absolutely with our recent findings in Bengal.

But let us not confuse things over such a slender thread of coincidence; the discovery of the existence of a cutaneous form of rinderpest in Bengal is unquestionably new and is reported here for the first time, but that by no means infers that this form has only just arisen. It has clearly been going on for centuries, as in Russia presumably, but has only been brought to light today in this country, being on the wane in its percentage of incidence as compared possibly with that of a thousand years ago. Even today in Bengal, the number of cattle in an average outbreak of rinderpest showing cutaneous lesions may be from nil to 30 per cent; more often it is from 5 per cent to 10 per cent. It is therefore,



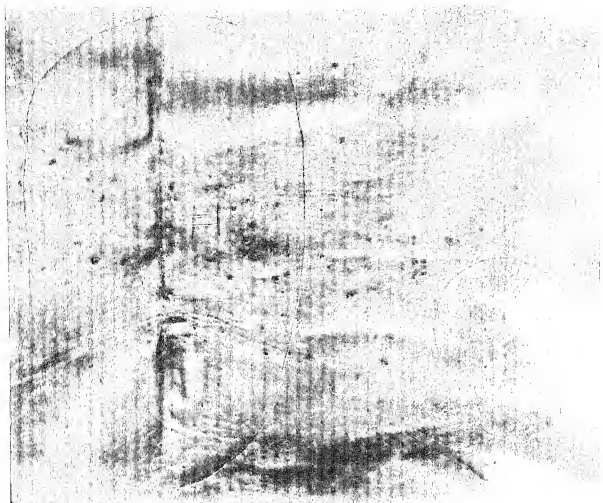
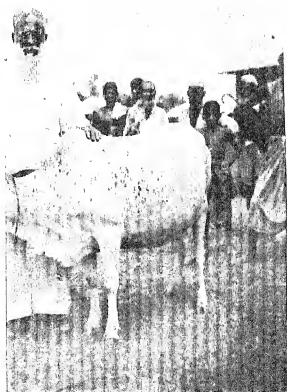


FIG. 1. Dark spots indicate nodules



FIGS. 2 and 3. Cases of Rinderpest showing skin lesions.

for other workers in India to attempt a closer observation for clinical evidence of this phenomenon than has been applied in the past; for it may be accepted that a condition of this kind, which has been lying in apparent obscurity for some 50 to 100 years, is not as obvious as the proverbial haystack. It requires at times painstaking search to discover the primary and even the later nodules, which, when burst, form a sort of matted scab of no particular colour other than that of the surrounding hair. The affected sites and their appearance are probably better studied from the accompanying photographs, (Plate IX) although most of these are of animals showing a much more prolific cutaneous affection than usual. In most outbreaks the number of clearly defined nodules or pustules has ranged between 10 and 100 over the neck, withers, flanks and/or lumbar region. Experimentally, the evidence on this point is meagre and not very substantial yet, as only one experimental calf showed three initial nodules on the neck some 14 days after inoculation with an emulsion of the scab from a field case of the disease, after developing an ordinary mild attack of alimentary rinderpest with perfectly typical mouth lesions. So that the exact period of development of cutaneous lesions after infection is not yet established; it probably approximates from seven to ten days.

However, certain other facts do stand out in the preliminary work on this series of incidents:

- (1) the scabs taken from the nodules of field cases are infective and capable, when emulsified, of reproducing rinderpest (experimentally in three instances) and even (in one case) of repeating what appeared to be cutaneous nodules resulting in scab formation;
- (2) these scabs were capable of reproducing true rinderpest even after some three weeks from the date of their removal from the animals in the field;
- (3) the blood from the calves so infected has been found capable of reproducing rinderpest in other calves experimentally (once confirmed by Mukteswar and twice by ourselves in Calcutta); and
- (4) this cutaneous rinderpest eruption, as it may be called, is the undoubted cause of a permanent pitting of the skin (shallow saucer-like depressions) which shows up prominently in the treated hide, to the extent of reducing the value by 40 per cent or more. In the finished hide, these depressions measure, when single, the diameter of an ordinary green-pea, but quite frequently one, two or even more become confluent, when the

damage to the hide is correspondingly increased. Admittedly, the guilt attaching to this cutaneous eruption as the cause of such depreciation in hide values was only proved in one case, in which the animal affected was slaughtered and the hide subjected by stages to tanning processes, after careful mapping of the original sites of the pustules. The depressions in the finished hide corresponded exactly with the pustule sites. But there is little reason to doubt that the same results will be obtained on repetition of the experiment, which was so carefully done with cooperation of Mr Das Gupta, M.Sc., of the Bengal Tanning Institute.

DISCUSSION

It is suggested that at this stage of the work on what appears still to be an insignificant problem, it is not wise to draw conclusions, but it cannot be denied that certain possibilities emerge from the material facts now available. It may be, for instance, quite reasonably held that where cutaneous rinderpest prevails the concepts of old regarding the spread and unaccountable recrudescence of rinderpest in areas previously presumed to be free require some form of reorientation; that the mystery of the so-called spontaneous rinderpest, so long unsolved as to its source, is now possibly given its conge; and that the economic importance of rinderpest does not end with the incapacity, or even with the death of the affected animal, but permeates the hide industry a most important threat to the country. Finally, it seems necessary to add that, from the obviously prolonged viability of the virus in these cutaneous scabs, their potential danger is difficult to exaggerate, not only from the very common and customary habit cattle have of scratching one another with their mouths and thereby ingesting in this way the scabs carrying the living virus, but also from swallowing during grazing scabs which have dried *in situ* and later fallen on to the pastures.

However, the presumptive evidence of this kind is all we have at present to support the contention referred to above that cutaneous rinderpest may be fraught with all-important repercussions, such as of explaining the so-called flare-ups of the disease in apparently free areas, or even of the rather unbelievable possibility of recovered animals being so-called carriers or rather vehicles through their scabs of the disease. Still, even after allowing that all things are possible, it seems reasonable to expect that where cutaneous rinderpest occurs, a higher degree of immunity among such local cattle should prevail; for it

must be remembered that to the cultivators even the appearance of cutaneous lesions in cases of rinderpest is the signal of recovery; the percentage of mortality in animals showing cutaneous eruption is very low indeed.

SUMMARY

In India and some other eastern countries, the name 'pox' is given to rinderpest, indicating a cutaneous eruptive fever, other than a severe inflammatory disease of the alimentary tract. In Bengal a benign type of rinderpest showing cutaneous lesions has been recognized; and sometimes 5-10 per cent of affected cattle exhibit from 10 to 1000 nodules, which eventually, with the progress of the disease, turn into scabs. Scabs from affected animals are capable, on inoculation and sub-inoculation, of producing rinderpest both of simple and cutaneous types. The prolonged viability of the virus in the dried scab seems to answer many of the problems relating to the appearance of so-called 'spontaneous' rinderpest. The cutaneous eruptions cause a great deal of damage to the hide industry and reduce the value of hides to the extent of 40 per cent.

ACKNOWLEDGEMENTS

Even a preliminary note of this kind would not be complete without acknowledging the work of collaborators.

Mr M. B. Menon, Superintendent, Veterinary Vaccine Section, was concerned with reproducing rinderpest in calves from emulsions of cutaneous scabs in two instances. The blood of one of these cases was confirmed at Mukteswar to be infective, although scabs from the same lot which produced the disease failed at Mukteswar to produce a reaction. This latter fact can only be explained as due to the loss of viability in transit or the age factor of the scabs.

Mr R. N. Mohan, Disease Investigation Officer, Bengal succeeded in his first attempt in reproducing typical rinderpest in at least one of two calves injected with scab emulsion. Blood from this infected calf was again successful in producing rinderpest in one of two calves sub-inoculated. In addition, calf first mentioned developed on the 14th day after injection of scab emulsion three nodules on the skin of the neck which resembled to a great extent those usually to be seen in field cases.

In respect of these latter experiments by Mr R. N. Mohan I cannot but praise the meticulous care and attention with which he conducted the work; he is, therefore, deserving of any credit which may emanate from this preliminary record.

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A STUDY OF SOME SIMPLE METHODS FOR DETECTING ADULTERATION IN GHEE

By NOSHIR N. DASTUR, D. R. KASHYAP and ZAL R. KOTHAVALLA, Imperial Dairy Research Institute Bangalore

(Received for publication on 31 May 1943)

The following studies were made to investigate the possible use of simple methods for differentiation between pure ghee and other edible fats, especially edible hydrogenated fats (*vanaspati*).

TILE TEST FOR GHEE

Sometime ago a method of detecting adulteration of ghee, henceforth to be called the 'Tile' test received publicity in the press. The following was the method advocated for carrying out the test. A small quantity of ghee (a drop or two), previously warmed, was to be applied on a baked earthen surface provided by either a country tile or a piece

of earthen pot. It was claimed for this method that, if the sample of ghee was adulterated with *vanaspati*, a white chalky deposit was left on the spot. This would not ordinarily happen with genuine ghee.

From a series of preliminary trials carried out it was found that porous, evenly smooth, unglazed, earthenware surfaces gave the best results. The fat to be tested was usually spread lightly on the surface or rubbed hard for 15 seconds. It was found that these two methods of applying fat did not make any difference in the results of the test. The test was always carried out at room temperature, i.e. no attempt was made to melt the fat.

The results obtained with the Tile test using different types of fats are briefly summarized as follows :

(a) *Vegetable oils.* All the edible oils tested gave negative results, i.e. left no chalky mark on the surfaces tried.

(b) *Vanaspati samples.* In all 25 different brands of *vanaspati* were examined. It was found that, with a single exception, all the samples left a chalky mark on the tile. It was also noticed that this particular sample which gave the negative result was quite hard compared with the other samples of *vanaspati*, which were in a semisolid condition at room temperature.

(c) *Animal depot fats.* These fats were extracted from the connecting tissues by the application of heat. Cow's and goat's depot fats gave a chalky deposit, while buffalo's and pig's fats showed negative results. It may be added that at room temperature, the buffalo fat was found to be as hard as either cow or goat fat.

(d) *Ghee samples.* In all about hundred samples of ghee were examined by the 'Tile' test. Samples were collected from almost all parts of the country with great care to ensure their genuineness. For this test, the direct use of butter instead of ghee made no difference as to its sensitivity. It was found that approximately 45 per cent of these ghee samples gave a positive result. The rest were negative. This serious defect indicates the unreliability of the test under everyday conditions.

(e) *Effect of heating the fats on the tile test.* It was found that when *vanaspati* samples which gave a positive 'Tile' test were heated to 60° C. and higher, they lost the property of throwing the chalky deposit on the tiles. Melted samples were always allowed to cool to room temperature by leaving them overnight, and in some cases were even solidified by keeping them in a refrigerator before testing. This behaviour of *vanaspati* is a great handicap in the application of the Tile test.

(f) *Detection of adulteration using tile test.* In view of the fact that quite a large number of genuine ghee samples gave a positive result, the utility of this method for the detection of adulteration of ghee, therefore, becomes very doubtful.

These results suggest that the positive results given by most of the *vanaspati* samples and other fats is due to the peculiar physical condition at the time of their examination rather than to any inherent characteristic.

PRESENCE OF NICKEL IN VANASPATI

A study to estimate the nickel content of *vanaspati* was undertaken. Any results of a similar study, if carried out in India, have not been

published. If nickel is found to be present in *vanaspati* in considerable proportions due to faulty methods employed in its manufacture, the test could then be utilized for the detection of such *vanaspati* if added to ghee.

For detecting the presence of nickel, an acid extract of the fats under test was prepared by the method recommended by Atack [1913] using Fortini's reagent as follows : 50 gm. of fat were weighed in a flask and 20 ml. of concentrated HCl added to it. The mixture was heated with frequent shaking and then allowed to stand. The

TABLE I

Samples.	Beller-Kries reaction		Remarks.
	Upper layer (ether)	Lower layer (acid)	
Vanaspati No. 1	Brown	Colourless	
" 2	"	"	
" 3	"	"	
" 4	"	"	
" 5	"	"	
" 6	"	"	
" 7	Pink	Pink	
" 8	Brown	Colourless	
" 9	"	"	
" 10	"	"	The lower layer changed to deep red after some time.
" 11	"	"	
" 12	Turbid	"	
" 13	Brown	"	
" 14	Colourless	Faint pink	The lower layer changed to reddish brown.
Vanaspati No. 15	Colourless	Colourless	
" 16	"	"	
" 17	Pink	Pink	
" 18	"	"	
" 19	Brown	Colourless	
" 20	"	"	
" 21	"	"	
" 22	"	"	
" 23	Brown	Colourless	
" 24	Yellow	Yellow	
<i>Ghee</i>			
Bombay	Pink	Brown	
Bombay	Faint pink	Yellow	
Jubbulpore	Pink	Brown	
Jodhpur	Colourless	Colourless	The upper layer changed to faint pink and the lower to yellow.
Jodhpur	Pink	Faint pink	
Ajmer	Faint pink	"	
Bangur	"	Yellow	
Darbhanga	Brown	"	
Darbhanga	"	Brown	
Calcutta	"	"	
Calcutta	Faint pink	Yellow	
Calcutta	"	Colourless	
Calcutta	Colourless	Yellow	
Narnaul	Faint pink	"	
Bangalore	Faint pink	"	
(Quilao)	"	"	
Bangalore (cow)	Colourless	Colourless	
Mandya	Faint brown	Yellow	

clear acid layer was pipetted in a silica basin, acid evaporated and the residue ashed in a muffle furnace at low red heat. After cooling, 1 ml. of Fortini's reagent was put over the ash and the development of colour noted. The silica dish was covered and left for 24 hours to note any development of the characteristic pink colour.

Fortini's reagent as used in the above tests was made by adding an equal volume of liquor ammonia to a saturated solution of dimethylglyoxine in absolute alcohol.

All the samples of *vanaspoti* tested by this method gave a negative result for nickel.

BELLIER-KRIES TEST

This test was carried out with fat samples of different origins to see if there was a clear demarcation between ghee and other fats.

The test was carried out by shaking 5 ml. of the liquid fat with 5 ml. of concentrated, colourless, nitric acid (sp. gr. 1.4) and 5 ml. of a 0.1 per cent

solution of phloroglucinol in ether. The mixture was shaken and allowed to separate in two layers. Results for ghee and *vanaspoti* samples are given in Table I.

These results show that there is no sharp demarcation between the colour shown by ghee and *vanaspoti*. Hence, the possibility of devising a test for differentiating between *vanaspoti* and ghee on the basis of the Bellier-Kries reaction seems very remote.

CONCLUSIONS

The 'Tilo' test, Dimethylglyoxine test for nickel and Bellier-Kries reaction do not make a sharp differentiation between ghee and *vanaspoti*. Hence, they cannot be employed for detecting adulteration in ghee.

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SOME NEW RECORDS OF NEMATODE WORMS FROM INDIAN RUMINANTS

By M. M. SAEWAR, L.V.P., Research Assistant, Imperial Veterinary Research Institute
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(Received for publication on 28 June 1943)

THE worms under record were collected on *post mortem* from a four-months-old local dairy calf of Hriana breed, a one-year old buffalo calf recently purchased from a village in the Bareilly district and a sheep that died naturally at Izatnagar. The dairy calf harboured more than 5,000 worms of the species, *Mecistocirrus digitatus*, *Cooperia punctata* and *Cooperia pectinata*. *Mecistocirrus digitatus* is by far the most common worm infesting cattle in India and is considered to be responsible for parasitic gastritis in calves. Sheather [1919] described what was evidently this species and considered it responsible for deaths among calves in a dairy herd. The calf under consideration had been dosed thrice with copper sulphate and iron sulphate during its illness which lasted about a month. The worms when fresh were dark-brown in colour, and under the microscope their intestines were found tinged greenish-blue, the worms on the whole presenting an unusual appearance. This was probably due to the drugs administered to the calf.

Cooperia pectinata and *Cooperia punctata* were reported for the first time by Rao [1940] from

south India. Bhalariao [1942] reported them from a hill-bull at Mukteswar. The present findings are recorded from plains cattle in northern India. The high infestation in the locally-bred calf and the presence of *C. punctata* in the buffalo calf, from the same district suggest the possibility of considerable infestations of cattle with these worms.

C. punctata, *Paracooperia nodulosa*, *Bunostomum phlebotomum* and a *Capillaria* sp. were obtained from the buffalo calf, and none of them has previously been recorded from buffaloes. *Oesophagostomum indicum* was collected from the sheep referred to above.

Paracooperia nodulosa [Schwartz 1929]

Schwartz [1929] described a parasite from the intestines of a carabao (*Bubalus bubalis*) which he called *Cooperia nodulosa*. The material was sent to him from the Philippines by Dr Gomez with the information that the animal was extremely emaciated and had died of inanition. On *post mortem*, the small intestines were found to contain nodules throughout its length and the teased preparations revealed round worms.

Schwartz described the nodules as conspicuously-raised bodies, varying from about 3 to 5 mm. in diameter, the summit of each nodule being more or less depressed and containing a small opening. Each nodule contained a single worm which was somewhat deeply embedded in the mucosa. On this evidence, he stated that the worm should be regarded as pathogenic and possibly of economic importance.

The intestines of the calf was found studded with nodules resembling those described by Schwartz, their number being about 20 per foot of the intestines. The nodules presented a greenish appearance when fresh.

The description of the parasite is based on individuals obtained free in the lumen of the intestines.

Travassos [1937] transferred the species *C. nodulosa* to his newly created genus *Paracooperia*. The following points have been studied from 10 individuals collected.

The head, varying in diameter from 0.06 to 0.065 mm. bears the papillae described by Schwartz. The oesophagus varies in length from 0.3 to 0.45 mm. and has a diameter of 0.3 to 0.45 mm. at its greatest breadth.

The male is about 9 mm. long and has a diameter of about 0.14 mm. at the anterior ends of the spicules. The appearance of the bursal rays is in agreement with Schwartz' description, save that the dorsal ray does not always present a bifid appearance at its branches. In one individual the right branch of the dorsal ray, besides giving off the lateral branch, bears a ray-like structure in the angle formed by the origin of the lateral branch. This structure is absent in the corresponding left branch, while the main branch has a bifid appearance. The spicules are from 0.25 mm. to 0.28 mm. long, differing in this respect from 0.304—0.32 mm. described by Schwartz, and bear about ten cusps each on their median process. The process bearing the cusps arises about 0.04 mm. from the anterior end of the spicule.

Females measure from 10 to 13 mm. in length and have a width of about 0.2 mm. in the region of the vulva. The vulva is situated about 2.5 mm. from the tip of the tail and is covered by a prominent cuticular linguiform flap. The combined length of the ovipositors, including the sphincters, is 0.8 mm. The tail, bearing alae, has a length of about 0.15 mm. The eggs are 0.07 to 0.075 mm. long and 0.049 to 0.054 mm. broad.

Host. *Bos bubalis*

Location. The small intestines; free or in nodules in mucosa

Locality. Bareilly district (U.P.)

Bunostomum phlebotomum Railliet, 1900

Host. *Bos bubalis*

Location. Small intestines

Locality. Bareilly district (U.P.)

Cooperia punctata (V Linstow, 1907)

Host. *Bos bubalis* and *Bos indicus*

Location. Abomasum and small intestines

Locality. Bareilly district (U.P.)

Cooperia pectinata down Ransom, 1907

Host. *Bos indicus*

Location. Abomasum

Locality. Izatnagar (U.P.)

Capillaria sp.

Only fragments of this worm were collected and identification was not possible. The eggs measure 0.048 to 0.05 mm. long and 0.0165 to 0.017 mm. broad.

Host. *Bos bubalis*

Location. Small intestines

Locality. Bareilly district (U.P.)

Oesophagostomum indicum [Maplestone, 1931]

The species was originally described from the large intestines of a spotted deer and a red deer in the Zoological Gardens, Calcutta. The two male oesophagostomes in the writer's material have a striking resemblance to the species under record, the only difference being the location of the cervical papillae. The papillae in the male are situated half way between the cephalic groove and the end of the oesophagus and not behind the oesophagus as described by Maplestone [1931].

The males measure about 13 mm. in length and have a breadth of about 0.3 mm. at the anterior end of the spicules. There is a cephalic swelling, with the groove situated about 0.2 mm. from the anterior end. Each of the leaf crowns contains ten broad elements. The spicules measure about 1.55 mm. long, and the accessory piece is about 0.065 mm.

Host. Sheep (*Ovis aries*)

Location. Large intestines

Locality. Izatnagar (U.P.)

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THE EXPLOITATION OF STRAW FOR FOOD

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IN normal times, or at least in times of peace cereal straws are used as food for animals to an important extent only in the backward agricultural areas of the world; elsewhere the main function of straw in agricultural economy is to form a basis for the production of manure. It thus happens that those who are the chief users of straw as forage are in no position to exploit modern knowledge in an endeavour to realize its full potentialities, while to those who could do so the subject generally is of little importance because they have easy access to richer sources of nourishment. When these sources are denied to them and when perhaps because of difficulties of transport and an urgent

demand for fats, the handling of material with a high oil and protein content is undertaken more readily than is that of the more carbonaceous foods, then the latter, being at hand, has its value much enhanced, while the deficiency of protein with which it may be associated and which in normal times makes it economically worthless, is then of less moment. It is, in fact, such conditions, or the possibility of such conditions, that have turned the attention of nutritionists to a study of the means of extracting from straw its maximum use as food.

A glance at the table below is sufficient to show the nature of the problem involved:

Composition of the cereal straws

Straw	Chemical composition				Digestible constituents				Ash	Starch equiva- lent
	Pro- tein	Oil	Carbo- hydrates		Pro- tein	Oil	Carbo- hydrates			
			Solu- ble	Fibre			Solu- ble	Fibre		
Oats (Winter)	1.9	1.5	43.1	34.6	0.5	0.5	19.8	19.7	4.9	21
Oats (Spring)	2.9	1.9	42.4	33.9	0.9	0.6	19.4	18.3	4.0	20
Barley	3.3	1.8	42.4	33.9	0.7	0.6	22.5	18.3	4.6	23
Wheat (Winter)	2.1	1.3	40.7	36.6	0.5	0.4	15.0	18.3	5.3	13
Wheat (Spring)	2.9	1.3	39.8	35.9	0.5	0.4	14.7	18.0	6.1	13

First, one sees how comparatively rich the straws are in carbohydrates but how poorly these carbohydrates are utilized by the animal and how that low digestibility applies not only to fibre but also to the soluble carbohydrates. Again, one sees immediately that little of the protein and fat is digestible, but that at any rate the quantity of these constituents is so small that they have little significance from a feeding point of view. The amount of ash is of some importance and more important still is the amount of silica which it contains. Although the total amount of minerals in straw is somewhat less than that contained in hay, its nature is very different because silica generally forms a quarter to a third of the straw ash, that is 1 to 2 per cent of the total composition, while the quantity of lime and phosphoric acid in it is negligible.

Straw thus can be made useful as a food only when the digestibility of the carbohydrates can be very considerably raised. To do that it is necessary to appreciate the conditions which account for their low digestibility. In the first place, as the analysis shows, more than half of the carbohydrates is composed of crude fibre. This crude fibre consists primarily of cellulose and other complex polysaccharides, the most important of which are lignin and cutin. The lignin and cutin envelop and impregnate the cellulose, forming as it were a vegetable skeleton as well as a protective covering. The crux of the question lies in the manner in which these substances are dealt with in the intestinal tract, for if they are not broken down the material which they enfold must remain unaffected by the digestive juices.

Pure cellulose is comparatively well digested by herbivora but not so by other types of feeders because its digestion depends not upon the action of intestinal enzymes as does that of other carbohydrates but on the action of the enzymes of

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symbiotic micro-organisms which themselves are closely associated with the food. Thus the nature and number of cellulose-splitting organisms found in the intestine at any given time depends upon the extent to which plant food has formed part of the diet previously, and the extent of their action in a large measure depends upon the facilities offered for their contact with the food over a prolonged period such as occurs in the rumen of cattle and in the caecum and colon of the horse. If the cellulose is encrusted with such material as lignin, cutin, silica, etc., their insoluble nature prevents the action of either digestive juices or bacteria, making the digestion of cellulose impossible and denies, even to the ruminant, the use of the other nutrients contained in it.

One of the features of the ageing of all grasses is the proportionate increase in cellulose, and in the lignification of its cellulose characterized by the rigidity and brittleness of its stem, so that as the plant gains maturity the amount of lignocellulose composing it rapidly multiplies. The cereal grasses are no exception to the rule, and as it is so important in the harvesting of the grain that there should be no lodging of the plants, those varieties having straw of a stiff nature which can thus withstand rough weather conditions are favoured in most districts. By far the commonest factor governing the amount of lignification of straw, however, is the stage at which the crop is cut. In the straw of the unripe oat, for instance, the crude-fibre content may be as low as 20.5 per cent or even lower, but when the same straw has been cut in a completely matured condition the fibre may have almost doubled in amount and the lignin, which in the former condition may have been almost absent, will in the latter case be present in large amounts. The shorter the period of growth the less will the process of lignification have proceeded. Of cereals, only oats are habitually cut in the unripe state, and it is for this reason that oat straw can without treatment form a useful food even for horses. On analysis, barley straw may appear to be superior to oat straw, but it is less palatable; wheat straw and rye straw are very much inferior and are useless as a food for horses—in fact, as is well known, the horse may expend more energy in dealing with such straw than is supplied by the nutrients absorbed from it. Their inferiority is almost entirely due to the indigestible nature of their carbohydrates.

Knowledge is still lacking as regards the exact nature of the association of lignin and cellulose. By some it is held to be a loose chemical combination; by others the relationship is considered to be a very close chemical one. The latter view is to-day more widely held than the former. Nearly all authorities are agreed that a characteristic of lignin is the presence of methoxy and acetyl groups

in its molecule, and it is known that these are easily split off by the action of heat and alkali and that if the process is repeated sufficiently often at high enough temperatures the lignin can be completely removed. Lignin and ligno-cellulose, the term used to denote the connection between the two substances, are quite indigestible, for they are acted upon neither by the digestive juices nor by the intestinal micro-organisms. It has been suggested and it can quite readily be understood that the indigestibility of fibre may not be so much dependent upon the amount of lignin which it may contain as the manner of the disposition of the lignin within the cell wall. The presence of but a small amount of lignin may or may not be responsible for a pronounced lowering of the digestibility of the fibre of a crop, depending on whether the lignin is distributed in a thin impervious layer locking up the otherwise digestible material or whether, on the other hand, the lignin is less evenly distributed and a certain amount of the fibre cells are free for digestion.

The common farm process which has been practised for generations of chopping, grinding or fermenting straw does only to a slight extent increase its digestibility. The chopping or grinding saves the expenditure of energy and to that extent enhances the feeding value of the material, the fermentation increases the palatability, but grinding may actually decrease it. What might be considered an extension of these processes carried out on a commercial scale has been tried in several countries. The best known one in this country was that carried out by the d'Orby Pulp & Fodder Company whereby the material was exposed to steam under pressure, dried and ground to a meal. The principle of a process used by Price in Australia was similar, while modifications of the same process were tried out in Germany and Russia. One may say, however, that, while palatability was greatly increased, in each case the food value of the straw was not sufficiently raised to make the process of much practical value.

Some fifty years ago Lehmann demonstrated that the nutritive value of straw could be enhanced by the action of hot alkali, and shortly afterwards Kellner showed that straw treated for the manufacture of paper by hot soda solution under pressure greatly increased its value as a food. Confirmatory tests in later years showed that anything up to 100 per cent of the cellulose freed from encrusting substances in this manner was digested by ruminants. Although straw was little used in Britain for paper-making, it was always used extensively for that purpose on the Continent.

When the process employed in paper manufacture was simplified to bring it within the range of ordinary farm practice, it was shown that the difficulties were great and the disadvantages very

real. Costly equipment was needed and a level of intelligence required for its application which precluded its use by other than at least semi-skilled labour. At a specialized central depot, however, where a plant was installed which was capable of dealing with large quantities, a material of high feeding value was produced at a cost which was not unduly great, that is when the treated straw or 'straw pulp' was used in its wet state. But therein lay the difficulty, because the water content of the pulp is very high, the transport of water is expensive, the process of drying is costly, and the semi-dried material is a favourable medium for the growth of moulds. The straw could then be used only in an area in proximity to the plant, in order to avoid the difficulties of transport and storage. The usefulness of this method of treating straw under factory conditions was thus greatly restricted, but, all the same, during the last European War many large-scale plants were installed in Germany.

As a result of the intensive study of the subject which was undertaken at that time, it was found that there was no necessity to carry the digestion of straw to the extent done in the paper industry. There the complete removal of the indigestible enveloping substances is aimed at and is achieved only at the expense of large amounts of organic material or potential food. Fairly strong concentrations of alkali and pressure steaming are the means adopted to that end. To greatly increase the food value of straw it is sufficient to disrupt the incrusting material and to spring the ligno-cellulose bond; in the process, the silica may be separated and dissolved and the methoxy and acetyl group split off the lignin molecule. It was discovered that this could be attained quite well when weak solutions of caustic were used, and it was also demonstrated that steaming at high pressure was not necessarily beneficial. Thus the expensive high-pressure process was discarded in favour of the use of weak alkali solution at low pressure. The resulting product is of an acid or neutral reaction because sufficient acetic acid is formed from the liberated acetyl group to neutralize the comparatively small amount of alkali. The process was in the meantime carried a step farther when cooking in open vats was advocated. A stronger solution of caustic soda was, however, employed and the treated straw had to be freed from alkali by washing before it could be fed. In washing there was an unavoidable loss of organic material which went far to offset the benefits which the simplified process otherwise afforded.

At Leeds in 1917 Godden, making use of the lessons already learnt on the Continent, devised a simple process which could be used on the farm. He recommended that chopped straw should be soaked overnight in a 1.5 per cent solution of

caustic soda, and on removal from the solution it should be well drained and put into a steamer, such as forms a fairly common piece of equipment on a modern farm. The particular one he devised consisted of a vertical iron boiler with a loose cover and fitted with a pipe which delivered steam near the base. Steam was blown through the soaked straw in this tank until the whole mass was at boiling-point, steaming being continued for an hour. The treated straw was allowed to drain and cool before being fed. It required no washing, because the soda had been sufficiently neutralized during the steaming. He showed that there was, on the whole, about 50 per cent gain in feeding value.

While Godden was doing this work in England, Beckmann introduced his method which differed radically from all the previous ones in that it did not involve the use of heat. He proved, too, that even under such conditions a weak solution of alkali was as effective for the purpose in hand as a strong solution and that the time required for its action was comparatively short. In spite of the fact that by his process final washing of the straw is required, the method is so simple and so economical that it quickly superseded all the others. The hydrolysis of the straw is carried out with eight times its weight of 1.5 per cent solution of caustic soda for three hours, the fluid is then run off and the residue washed with water until it is neutral to litmus. The results of feeding trials, digestibility and metabolism experiments show that, in general, for ruminants, the nature of the improvement in food value is in the region of 100 per cent.

Hydrolysis of straw can be accomplished with material other than caustic soda—such, for instance, as calcium hydroxide and sodium carbonate, or by acids such as hydrochloric, sulphuric or nitric. Lime and sodium carbonate are considerably less effective than sodium hydroxide unless heat is used, when their action compares very favourably with that of caustic soda. The action of the acids is not sufficient in any case to make their use worth while.

Today Beckmann's process is being experimentally operated on many farms in Britain, and feeding trials on ruminants are also being conducted under the auspices of the Agricultural Research Council. The result of these trials and in particular the reports on the suitability of the process to English farm conditions may be of some importance. The plant most generally adopted is one of two devised by Imperial Chemical Industries, Ltd.

One of these consists of twin concrete basins into either of which is placed the chopped straw, both while it is being subjected to the action of the caustic soda and while it is being washed. After the straw has soaked sufficiently in a 1.25 per cent

sodium hydroxide solution, a volume of fresh water is run on to it until the basin is brimful. The heavy caustic solution lies practically undiluted at the bottom of the basin and is now by a simple but ingenious system of piping syphoned off to the neighbouring basin for use on the next consignment of straw, while the head of water which has permitted the syphoning of the soda is diverted to an outside channel as is the water with which the straw continues to be washed until it has lost all feeling of soapiness. That process may take from one to three hours, depending upon the rate at which the water is being passed through. The straw is then fit for immediate feeding, but is usually allowed to drain for an hour or two in order to get rid of surplus fluid. The caustic solution which has been syphoned into the second basin is made up to the original strength and volume and the process is repeated.

The other type of plant is yet more simple, but involves a somewhat greater amount of work. It consists of two tanks separated by a drainage platform. After the chopped straw has been soaked in one tank it is laded on to the platform from where the surplus lye runs back to the tank from where it was lifted. The straw is then shovelled into the second tank, where it is washed by successive steepings in water or by a continuous stream of water. Again, it is generally found to be advisable to drain the surplus liquid from the straw before feeding. In either plant there is some difficulty experienced in the initial soaking, but that is overcome by means of sectional wooden frames over which half-inch wire netting is spread. These are placed on the straw, and with the help of heavy concrete weights they press the straw down into the solution.

The action of the caustic solution on the straw is most intensive for a comparatively short period during the first hour or two. Beckmann himself considered that three hours was the optimum time for soaking, but the process of digestion actually continues after the first three hours, although progress then is very slow. In practice it often proves convenient to soak the straw overnight and wash it first thing in the morning. The process will certainly have gone as far as desirable by twenty hours with the temperature at 45°F., but if the weather is cold and the temperature at, say, freezing-point, the straw must be exposed to the action of the salt for very much longer if the same degree of digestion is to be attained. As indicated, however, something considerably short of twenty hours is sufficient for practical purposes.

The amount of water required for washing is about four gallons for each pound of straw. The time required to handle 200 lb. of straw by one man is three-quarters of an hour. The all-in cost

of the treatment at present prices in Britain is in the region of £3 per ton.

The straw is generally fed in the wet state and when stock have once become used to it they eat it readily. Some animals take to it at once, but other individuals come to it more slowly. We have, however, seen cattle, coming straight from good grazing, accept the pulped straw almost immediately, and we have seen horses accustomed to good rye-grass hay readily accept the treated straw in its place. There is, however, no reason on the score of palatability why the straw should not be dried and stored. During the drying process, however, when the material is damp rather than wet, it is liable to ferment and become musty, but once that stage, so favourable to the growth of moulds, has passed the treated straw will keep as well as it did before treatment.

As already indicated, the treatment more or less doubles the starch equivalent value of the straw for ruminants—that is, it brings the energy-producing value of oat straw up to level of that of the cereals, and of wheat straw to that of really good hay. It is probable that for horses the value of the straw is raised relatively more than it is for ruminants; in fact, a digestibility experiment with these animals showed that a poor barley straw with a starch equivalent as low as 5.5 per cent was raised to an equivalent of 20.2 per cent which brings it to the comparative value of poor hay. As a food, however, its almost complete lack of protein must be remembered and that deficiency must be made good by a supplement. In Germany in [Fingerling 1917] horses fed solely on such a mixture maintained good condition while being subjected to hard work, while in 1919 hydrolized wood meal with a protein supplement was substituted for hay and even for oats with satisfactory results. [Ellenberger 1919] We have found that resting horses maintained their condition over a period of four months during which caustic-treated straw took the place of hay.

So far as we know, progress in the methods of treating straw on a farm scale so as to overcome its natural indigestibility rests at the stage to which Beckmann brought it. Some refinements have been added, but the principles remain substantially unaltered. In practice, there are many difficulties connected with it, but most of them have been exaggerated in prospect and have disappeared on acquaintance. One unaccustomed to the work would immediately question the advisability of allowing the free use of such potentially dangerous material as caustic soda, both to humans and to stock, but a few simple precautions overcome the difficulty and no real objection to it is ever offered by those using the material. More serious objections are those made on economic grounds—the cost of soda, the necessity of washing and the

amount of water used, the cost of chopping the straw and the difficulty of handling such bulky material, the cost of the plant, etc. The answer to these objections lies either in the monetary value of the final product or in the necessity of producing the food irrespective of its cost. Let us say at once that it was probably the latter consideration which stimulated the intensive research on the subject which was carried out in Germany during the last war, and that even Beckmann's process is unlikely to prove economical in times of plenty, when natural highly nutritious forage is at hand in abundance. It is not difficult, however, to visualize conditions either in war or peace when the treatment of straw for food might become of great importance. A shortage of fodder may be secondary only in time of a shortage of food for man, and it is not an infrequent companion of an army, even a mechanized one—in fact, one may say that it is sooner or later inevitable in a war zone. But even in peace time the conditions following natural calamities may make it of vital importance that material on the spot should be exploited to the full. Even under normal conditions, such as apply in certain districts of Asia and Africa where straw is relied upon to a large extent to maintain an enormous head of cattle over the greater part of the year, a process of treatment might find a place, as that of Beckmann does in Europe.

In our own sphere is it not conceivable that an economic method might be evolved whereby the grass straw issued under the flattering term of 'hay' to the Army in India might have its value increased so that it did more accurately answer to its official designation? That the term 'grass straw' is more appropriate to such forage than 'hay' or 'grass hay' will be conceded by all those who are familiar with the methods by which

it is saved. If there should be any doubt as to its true nature the chemical analysis and the digestibility trials conducted by Lander [1937] will dispel it. Calculated on his data, the hay from military grass farms in the Punjab has the following composition:

Chemical analysis	Digestible nutrients
Crude protein	4.4 1.6
Fat	1.3 0.4
Soluble carbohydrates	38.21 18.36
Fibre	38.2 23.3
Ash	9.48

The starch equivalent of hay at eleven of the principal stations in India, according to Sen, [1933] is 21.5 per cent. The same author found wheat *bhoosa* to have a starch equivalent of 22.1 per cent. These figures apply to ruminants and their equivalent for horses is probably much lower.

It would, of course, be quite wrong to suggest that methods of treating straw which are applicable to temperate climates would also be suitable in the tropics. The shortage of water and often the shortage of fuel alone make conditions quite different, but, on the other hand, the density of animal population at certain centres, the necessity of maintaining them on straw throughout the year, and the abundance of labour, among other considerations, allow one to suppose that attempts to evolve a system suitable for such localities might be worthy of consideration.

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REPORT ON PHENOTHIAZINE AS AN ANTHELMINTIC IN HORSES*

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OBJECT OF INVESTIGATION

THE object of the investigation was to determine

- (1) The efficiency of the drug as an anthelmintic
- (2) A simple method of administration of the drug.
- (3) The reaction of the animal to the drug.
- (4) The minimum effective dose.

SELECTION OF ANIMALS

The first batch of horses examined had been in the barrack stables sometime prior to the outbreak of the war.

* An investigation conducted under the co-ordination of The Helminths Committee (Animals Section) of the Agricultural Research Council.

The subsequent examinations were made on remounts; these horses were from units in various parts of England and Scotland and the majority had been newly acquired by the Army.

Each animal was subjected to a routine examination and weighed, and special attention was paid to the teeth faeces and blood. The drug was not given to any animal that showed any deviation of temperature or pulse.

Apparent poor condition was not always an indication that the animal had a high infestation of helminths, and, conversely, neither was good condition invariably an indication of a low infestation. It was discovered that many of the

debilitated poor 'doers' had a low or almost negative count, whereas many animals which appeared to be in 'good condition' were heavily infested; these animals were treated.

Practically all the horses, whether in good or bad condition, improved and gained in weight following the administration of the drug.

All the animals chosen were infected with strongyles and occasionally with ascarids.

ADMINISTRATION OF THE DRUG

The data on the use of phenothiazine as an anthelmintic in the horse is somewhat scanty, and the dosage, method of administration, etc. required further investigation. The first doses given in the present series were based on 5 gm. per 50 lb. body weight, which usually worked out at approximately 100 gm. for the average cavalry horse.

One hundred gm. of phenothiazine is a bulky mass and its administration offered a problem. The powder is not miscible in water, but may be suspended and given by stomach tube.

The administration of the powder in the food was tried with success. The procedure was to feed a bran mash mixed with treacle, followed at the next meal by a similar feed with which the powder was mixed. Some animals reluctantly ate the mixture, while others, especially the 'particular feeder', left it. The following method was found to be the most satisfactory. The animals were made to miss a feed, and then an ordinary bran mash containing the powder was given. In almost every case the feed was eaten, and there was no difficulty when a little boiled oats was added.

It was the usual practice to treat the animals at the week-end, missing the Saturday morning feed and giving a meal containing the powder at the mid-day feed. In this manner the usual routine of work was not interfered with. The animals were at their usual work on Monday, having suffered no apparent inconvenience. However, to prevent the working of any horse that might be particularly sensitive to the action of the drug, each animal was examined every morning for a week subsequent to the administration of the medicine.

REACTIONS OF THE DRUG

Some interesting observations were made which seem to have a bearing upon the distribution and possible 'breakdown' of the drug in the body system, although some horses showed little or no departure from the normal.

(1) The conjunctival mucous membranes were stained a yellowish colour and occasionally the mucous membranes of the mouth and anus were

similarly coloured. The yellow coloration appeared after 24 hours and sometimes became more intense, but gradually faded out. In some, the coloration disappeared in a few days, but in others it persisted for seven to eight days and even longer. The mucous membranes of the mouth and anus were stained a faint yellow, very much less intense than that of the conjunctiva.

(2) The urine of some horses was viscid and coloured a reddish brown. Attention is drawn to this condition by the staining of the bedding and floor of the stable, and by the red-speckled hind limbs, especially noticed when the limbs have white markings.

The coloured excretions may persist for two to four days. In several animals, the urine remained coloured for several days before a clearance was effected.

Those unaware of this action of the drug might be alarmed and consider the reaction unfavourably. Analysis of the urine did not show any unusual ingredients, other than might be expected from the 'breakdown' of the drug.

Technique. To measure the anthelmintic efficacy of the drug, egg counts were carried out before and after dosing.

The modified stool dilution method was used throughout: 5 gm. of faeces were weighed out into a jar graduated at 75 c.c. and N.10 NaOH added up to the 75 c.c. mark. The sample was left overnight to soften, and after shaking and sieving to remove coarse debris, 0.15 c.c. was removed by means of a Macdonald pipette. All the eggs in the 0.15 c.c. were counted and the process was repeated three or four times, and the average count taken. The figure obtained, multiplied by 100, gave the number of eggs per gm. of the original sample. Eggs of *Parascaris equorum* were counted separately from those of strongyle worms. The faeces were taken direct from the rectum of each horse.

Explanation of Tables. Where a count of 30 eggs appears in the tables, this indicates that only one egg was seen in the three slides examined. A plus sign in the tables indicates that no eggs were found by the above counting technique, but that a concentration method using about 5 gm. of faeces revealed a few eggs.

Owing to the transfer of horses, some irregularity occurred in respect of the intervals of time between the three counts after dosing. In Tables I, II, III, VII and VIII, the counts were made at approximately two, four and six weeks after dosing, and in Tables IV, VI, IX and X at two, three and four weeks. In Table V, only one count was obtained, and this was taken at four weeks. In every case where a third count was obtained, it was taken at not less than four weeks after the date of dosing.

TABLE I

Results obtained from the administration of 100 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A38	11	1,000	0	0	330
A39	11	1,400	0	0	230
A42	6	850	0	0	+
A46	8	560	+	+	130
A47	8	1,000	0	0	60
A50	9	600	0	0	30
A52	6	1,050	0	0	60
A79	15	1,200	0	0	30
A86	11	750	0	0	360
A89	8	800	+	+	160
A91	6	800	+	+	+
A99	9	1,560	+	+	+
B40	10	600	0	0	0
B44	7	400	0	0	0
B46	9	1,060	+	30	60
B51	6	260	+	30	130
B54	8	1,330	0	+	30
B58	8	830	30	60	200
B59	8	1,400	0	0	+
B68	8	60	0	0	+
B69	6	1,160	0	0	+
B70	8	600	+	+	+
B72	7	600	0	0	60
B73	15	460	0	0	+
B76	14	1,780	0	0	260
B77	12	450	0	+	60
C60	14	1,300	230	200	260
C81	7	1,360	+	+	60
C86	15	1,430	+	+	30
C70	12	1,200	0	0	+
D2	8	630	0	30	130
D28	10	250	+	+	+
D36	6	1,000	+	+	100
D45	9	970	0	0	+
t.b.d.	2	970	+	+	+

TABLE II

Results obtained from the administration of 95 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
C59	15	2,500	0	0	130
C72	9	1,030	0	0	+
C75	15	1,030	0	+	+

TABLE III

Results obtained from the administration of 80 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A44	7	960	0	0	+
B63	8	1,000	+	+	130
B75	6	560	+	+	+
B8	9	630	330	200	200
D49	10	700	0	0	230

TABLE IV

Results obtained from the administration of 4 fl. oz. Phenovis-72 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A7	10	1,200	0	0	0
A33	13	60	0	0	60
A48	6	1,300	+	+	+
A56	7	600	0	0	0
A59	13	300	0	0	+
A73	10	500	0	0	0
A75	8	800	0	0	0
A76	6	500	0	0	0
A80	15	830	0	0	0
A84	12	500	0	+	0
A92	12	560	0	0	+

TABLE V

Results obtained from the administration of 60 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.	
		Count before dosing	Count after dosing
B31	9	1,000	30
B35	15	300	160
B39	10	130	30
B41	8	700	0
B42	15	1,430	0
B43	9	660	30
B49	8	500	0
B52	6	730	30
B55	11	820	30
B57	14	800	60

TABLE VI

Results obtained from the administration of 3 fl. oz.
Phenovis-52 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A64	6	130	0	0	0
A72	8	560	0	0	0
B9	8	1,100	60	60	60
B13	13	800	0	0	0
B18	12	1,500	+	+	60
B21	8	300	+	+	+
B23	11	800	0	0	+
B27	15	1,430	0	0	0
B29	9	2,460	0	0	0
B37	12	890	+	30	30
D85	15	1,860	0	+	360

TABLE VII

Results obtained from the administration of 35 gm.
of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
D81	10	1,600	+	+	120
D83	8	2,060	320	520	1,120
D84	8	2,000	0	0	200
D86	7	1,520	0	0	60
D87	7	1,060	0	0	120

TABLE VIII

Results obtained from the administration of 30
gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
D90	8	1,400	0	0	120
D91	10	1,200	0	+	260
D92	7	1,000	0	0	Destroyed.
D93	11	1,120	0	60	120
C50	9	860	+	60	320

TABLE IX

Results obtained from the administration of 25
gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
C37	14	2,800	120	120	600
C65	7	2,000	400	520	720
C71	7	1,320	60	60	600
C72	19	1,400	320	320	400
C77	15	1,060	60	60	400

TABLE X

Results obtained from the administration of 20 gm.
of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
D49	10	1,400	320	460	660
C1	10	1,320	520	860	800
C15	6	860	400	520	460
C21	6	1,060	120	400	400
C24	7	1,200	60	60	60

TABLE XI

The following Table is compiled from the preceding
tables and gives egg counts for *Parascaris equorum*.

Horse No.	Age	Ascaris eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A42	6	330	0	0	0
A52	6	60	0	0	0
A64	6	30	+	+	30
A86	11	60	0	0	30
A91	6	30	0	0	0
B54	8	30	0	0	0
B59	8	60	0	0	0
B68	8	60	0	0	0
B77	12	30	130	60	0
D28	10	100	60	30	30
D36	6	50	100	100	30

TABLE I

Results obtained from the administration of 100 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A38	11	1,000	0	0	330
A39	11	1,400	0	0	230
A42	6	850	0	0	+
A46	8	560	+	+	130
A47	8	1,000	0	0	60
A50	9	600	0	0	30
A52	6	1,050	0	0	60
A79	15	1,200	0	0	30
A86	11	750	0	0	360
A89	8	800	+	+	160
A91	6	800	+	+	+
A99	9	1,560	+	+	+
B40	10	600	0	0	0
B44	7	400	0	0	0
B46	9	1,060	+	30	60
B51	6	260	+	30	130
B54	8	1,330	0	+	30
B58	8	830	30	60	260
B59	8	1,400	0	0	+
B68	8	60	0	0	0
B69	6	1,100	0	0	+
B70	8	600	+	+	+
B72	7	600	0	0	60
B73	15	460	0	0	+
B76	14	1,780	0	0	260
B77	12	450	0	+	60
C60	14	1,300	230	200	260
C61	7	1,360	+	+	60
C66	15	1,430	+	+	30
C70	12	1,200	0	0	+
D2	8	630	0	30	130
D28	10	250	+	+	+
D36	6	1,000	+	+	100
D45	9	970	0	0	+
t.b.d.	2	970	+	+	+

TABLE II

Results obtained from the administration of 95 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
C59	15	2,500	0	0	130
C72	9	1,030	0	0	+
C75	15	1,030	0	+	+

TABLE III

Results obtained from the administration of 80 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A44	7	960	0	0	+
B63	8	1,060	+	+	130
B75	6	560	+	+	+
B8	9	630	330	200	200
D49	10	700	0	0	230

TABLE IV

Results obtained from the administration of 4 fl. oz. Phenovis-72 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A7	10	1,200	0	0	0
A33	13	60	0	0	60
A48	6	1,300	+	+	+
A56	7	600	0	0	0
A59	13	300	0	0	+
A73	10	500	0	0	0
A75	8	800	0	0	0
A76	6	500	0	0	0
A80	15	830	0	0	0
A84	12	500	0	+	+
A92	12	560	0	0	0

TABLE V

Results obtained from the administration of 60 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.	
		Count before dosing	Count after dosing
B31	9	1,000	30
B35	15	300	160
B39	10	130	30
B41	8	700	0
B42	14	1,530	0
B43	9	660	30
B49	8	500	0
B52	6	730	30
B55	11	820	30
B57	14	800	60

TABLE VI

Results obtained from the administration of 3 fl. oz.
Phenovis-52 gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A64	6	130	0	0	0
A72	8	560	0	0	0
B9	8	1,100	60	60	60
B13	13	800	0	0	0
B18	12	1,500	+	+	60
B21	8	300	+	+	+
B23	11	800	0	0	+
B27	15	1,430	0	0	0
B29	9	2,460	0	0	0
B37	12	800	+	30	30
D85	15	1,860	0	+	360

TABLE VII

Results obtained from the administration of 35 gm.
of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
D81	10	1,600	+	+	120
D83	8	2,060	320	520	1,120
D84	8	2,000	0	0	200
D86	7	1,520	0	0	60
D87	7	1,060	0	0	120

TABLE VIII

Results obtained from the administration of 30
gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
D90	8	1,400	0	0	120
D91	10	1,200	0	+	260
D92	7	1,000	0	0	Destroy- ed.
D93	11	1,120	0	60	120
C50	9	860	+	60	320

TABLE IX

Results obtained from the administration of 25
gm. of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
C37	14	2,800	120	120	600
C65	7	2,000	400	520	720
C71	7	1,320	60	60	600
C72	10	1,400	320	320	400
C77	15	1,060	60	60	400

TABLE X

Results obtained from the administration of 20 gm.
of phenothiazine powder

Horse No.	Age	Strongyle eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
D49	10	1,400	320	460	600
C1	10	1,320	520	860	800
C15	6	860	400	520	460
C21	6	1,060	120	400	400
C24	7	1,200	60	60	60

TABLE XI

The following Table is compiled from the preceding
tables and gives egg counts for *Parascaris equorum*.

Horse No.	Age	Ascaris eggs per gm.			
		Count before dosing	1st count after dosing	2nd count after dosing	3rd count after dosing
A42	6	330	0	0	0
A52	6	60	0	0	0
A64	6	30	+	+	30
A86	11	60	0	0	30
A91	6	30	0	0	0
B54	8	30	0	0	0
B59	8	60	0	0	0
B68	8	60	0	0	0
B77	12	30	130	60	0
D28	10	100	60	30	30
D36	6	50	100	100	30

DISCUSSION

After doses ranging from 30 to 100 gm. the first two egg-counts were negligible, except in a very few cases. In the third count, on the other hand, a slight rise was found in the majority of cases. This figure however, is very small in comparison with the count before dosing and is no more than might be expected in view of the lapse of time between the times of dosing and the third count. The increase may be explained by the maturing of larvae which were in the tissues at the time of dosing, and which had returned to the lumen of the gut in the interval.

Doses of less than 30 gm. however, did not produce comparable results, although a notable reduction in the egg-count was recorded, for the 25 gm. group of approximately 80 per cent, and for the 20 gm. group of 40 per cent. In these two groups the second and third egg-counts show a rapid increase, and it would seem that while the drug did not kill all the worms, nevertheless it affected the egg-laying activities of the female, for a period of nearly three weeks.

On the evidence set forth in the above table the minimum effective dose is considered to be approximately 30 gm.

Only 11 horses of the total of 95 treated, harboured *Parascaris equorum*, and infestations were light in all cases. No definite conclusions can therefore be drawn from these observations concerning the value of the drug against this parasite, and although the egg-counts from six of the animals were negative after treatment, the other results were erratic.

CONCLUSIONS

(1) It would appear from the results given in the above tables of egg-counts and from the improvements observed in the general condition of the animals treated that phenothiazine has distinct merits as an anthelmintic for strongyles in horses.

(2) The average weight of the horses treated in the above experiment was approximately 1,000 lb., and the effective minimum dose was 30 gm. This represents 1 gm. of phenothiazine per 33 lb. of body weight.

(3) In view of the fact that phenothiazine may be given in the feed of the horse with little previous preparation of the animal it has obvious advantages over drugs requiring long preliminary fasting and more elaborate methods of administration.

(4) No evidence was obtained of any untoward reactions, and although slight malaise was observed in two or three animals, this may have been due to individual idiosyncrasy.

(5) The drug has the additional value that it does not interfere with the normal working of the horse.

(6) The foregoing conclusions suggest that regular periodic dosing with phenothiazine might provide a safe and easy method for the control, if not the complete eradication of strongyles in horses.

ACKNOWLEDGEMENTS

We desire to express our thanks to Lieut.-Col. C. G. Darley, D.S.O., Commanding Officer, 3rd horse Cavalry Training Regiment, and to Major D. A. Gilmor, R.A.V.C., for the facilities to carry out this investigation.

TREATMENT OF EQUINE STRONGYLOSIS BY PHENOTHIAZINE

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At the suggestion of E. L. Taylor, D.V.Sc. D.V.H., M.R.C.V.S., Senior Research Officer of the Laboratory of the Ministry of Agriculture, and Fisheries, Weybridge, it was decided to carry out trials at the Royal Army Veterinary Corps Laboratory to test the efficacy of the anthelmintic drug thiodiphenylamine, also known as phenothiazine, for the treatment of strongylosis in horses.

This drug has proved very effective against stomach worms in sheep and would appear to exert more effect than any preparation previously tried for the removal of worms from the large intestine of sheep. It was thought, therefore

that phenothiazine might prove an efficient expellant of strongyles from the horse.

Three horses were selected for these observations:

- (1) No. 110. Bay Mare, 7 years old. Rider. Condition moderate.
- (2) No. 47. Bay Mare, 7 years old. Rider. Condition moderate.
- (3) No. 94313. Black Mare, aged. Rider. Condition poor.

For the purpose of this trial egg counts of the faeces were made by Stoll's method before and after the treatment, and were continued for 21 days after administration of the drug. The results of these counts are shown in table on next page.

Record of treatment and egg counts

Subject	Date	Strongyle eggs per gm.	Treatment and remarks
Horse No.			
110	26-10-39	600	60 gms. phenothiazine in 1 gallon warm water per stomach tube. No parasites in faeces. <i>Strongylus edentatus</i> and <i>Strongylus vulgaris</i> present in faeces. Do. Do. Do. Do. Do. Do. No parasites in faeces.
	27-10-39	600	
	30-10-39	300	
	31-10-39	200	
	1-11-39	0	
	2-11-39	100	
	3-11-39	0	
	4-11-39	0	
	5-11-39	0	
	to		
	20-11-39		
Horse No.			
47	26-10-39	600	100 gms. phenothiazine in 1 gallon warm water per stomach tube. No parasites in faeces. Do. <i>S. edentatus</i> and <i>S. vulgaris</i> in faeces. No parasites in faeces. <i>S. edentatus</i> and <i>S. vulgaris</i> in faeces. No parasites in faeces.
	27-10-39	600	
	1-11-39	300	
	2-11-39	900	
	3-11-39	100	
	4-11-39	200	
	5-11-39	0	
	6-11-39	0	
	7-11-39	0	
	to		
	22-11-39		
Horse No.			
94313	14-11-39	100	60 gms. phenothiazine in food. No parasites in faeces. Do. <i>S. edentatus</i> , <i>S. vulgaris</i> and <i>Trichonema</i> in faeces. Do. Do. Do. Do. No parasites in faeces
	15-11-39	100	
	16-11-39	200	
	17-11-39	100	
	18-11-39	0	
	19-11-39	0	
	20-11-39	0	
	21-11-39	0	
	22-11-39	0	
	to		
	7-12-39		

From the result of the egg counts as shown in the above table it will be noted that before treatment none of the horses gave very high counts and these animals were not regarded as acute cases of strongylosis. They were selected as the most suitable cases at our limited disposal for this observation.

The phenothiazine was supplied by Dr. Taylor in tablet and in powder form. In two instances the drug was administered by stomach tube and in the third by mixing with the food. When the tablets were used these were finely ground before administration.

On mixing with water phenothiazine tends to sediment rather rapidly, but we found that by keeping the suspension constantly stirred during administration no sediment remained when given by the stomach tube. The drug was given in one gallon of warm water.

The other method of administration consisted of mixing the drug with food. For this purpose a quarter bucket of warm bran mash was prepared and the powder well mixed with it.

Although the mare selected for this trial had been fasted for 24 hours previously she did not regard the feed favourably, ate about a third of the mash and refused to take more. When, however, a double handful of oats was added to the remainder of the mash she rather reluctantly finished it.

It has been suggested that horses may be induced to take the drug more readily by giving them small amounts of treacle, bran and oats for a few days previously and then mixing the powdered drug with this food when it is readily taken. In further trials it is proposed to employ this method.

As far as dosage is concerned, two horses received 60 gm. and one 100 gm. of phenothiazine.

The smaller doses, judging from the egg counts, appeared to be just as effective as larger ones, but as we were only dealing with comparatively light infestations the same rule may not apply to heavy invasions.

No ill effects were noticed after administration, there was no softening of the faeces, no rise in temperature and the appetite was unaffected.

In each case three worm egg counts were carried out on separate days before administration.

Red-tinged urine was observed in each horse on the day after the administration of the drug. This pigmentation disappeared in about 48 hours. The red coloration is due, not to the presence of blood, but to a colour reaction derived from the drug.

The horses were prepared by fasting for 24 hours before administration, after which they received only warm bran mash for a further 24 hours, and then reverted to ordinary diet.

A naked-eye search for parasites was carried out on all dung passed after the drug was given. No special apparatus was employed for this purpose: the pellets merely being broken down and carefully examined. A few parasites were observed in all three cases, these appearing upon the second- or third day after administration and persisting until the fifth day.

Specimens were despatched for classification to Dr. Taylor, who identified them as *Strongylus edentatus*, *Strongylus vulgaris* and *Trichonema*.

In two of the horses two species of *Strongyles* were observed only, in the third horse all three species were present.

Egg counts were taken and observations were recorded for a period of 21 days after administration.

It will be seen from the tabulated record that in addition to the expulsion of adult worms the drug was completely effective in removing ova of *Strongylidae* from the faeces, as in the counts taken prior to administration eggs were present in, at any rate, appreciable numbers. It must be noted that in our previous experiences with the use of anthelmintic drugs for the treatment of strongylosis of horses, although a reduction in the number of ova in the faeces was brought about after a time the number again increased. It will, therefore, be necessary to make observations over a longer period than has been done in these experiments and to consider the advisability of giving a second or even a third dose of the drug.

As an anthelmintic for strongylidae in horses the results appear to be very encouraging and would justify the carrying out of trials upon a more extensive scale. In comparison with other anthelmintics used for the treatment of strongylosis in horses phenothiazine appears up to the present to have a decided advantage in being harmless to the patient.

The question as to whether or not a purgative could be usefully employed in combination with this drug is a matter for further consideration.

ABSTRACTS

Artificial insemination and the veterinary surgeon with special reference to cattle. N. J. SCORRIE (1943). *Veterinary Record* 55, 56

THE basic research leading to the modern development of artificial insemination was first initiated by Elick Ivenoff, a Russian physiologist in 1899, as a result of which active work on this branch of science in the field, which began in 1923, made it possible for breeding of millions of cattle, horses, goats and pigs in the U.S.S.R. Organized artificial breeding on large scale collective farms in the U.S.S.R. was largely facilitated by conditions peculiar to the Russian stock-raising system. As a result thereof in 1938, 50 million farm animals were inseminated which greatly speeded up livestock improvement and the introduction of new types. Similarly in Denmark, where the first co-operative breeding society was formed, progress was recorded and in Italy two institutions were set up by Government to study the technique of the operations and associated problems. In Kenya a rapid extension of this method of breeding took place in 1938-39 mainly with a view to lowering the prevalence of genital diseases transmitted by coitus. At the end of 1941, 86 farms, with a total number of 15,003 breeding females and 158 bulls, were practising artificial

breeding. These figures represented 23 per cent and 6.9 per cent of the total cow and bull population. The prompt and widespread adoption of insemination had saved the dairy industry serious loss from genital diseases. Out of these 36 farms, 24 farms involving 11,910 cows had adopted this practice because of the existence of infection in these herds, and the rest involving 3,093 cows, i.e., 20.6 per cent of the total, and 40 bulls representing 25.3 per cent for reasons of economy of bulls. On an average 1.88 inseminations were required per conception.

In the United States of America dairy cattle breeders have clearly recognized the economic benefits and other advantages of artificial breeding, and almost without exception the American Associations, which are 54 in number spread over 22 States, have arisen as a result of real demand for this service by breeders themselves.

The principal objects of artificial insemination are classed under (a) livestock improvement, (b) disease control and (c) breeding efficiency. Livestock improvement can be effected by the use of a limited number of first-class bulls available for mating with many more times the number of females than would be possible under natural conditions. For example, in Russia as many as 1,538 cows were inseminated from the semen of one bull in one year producing 1,490 calves, and 15,016 ewes were

similarly inseminated with successful results. First-class sires, in other words proven sires, whose ability to transmit desirable characters has been determined, are very few; for in dairy practice the majority of sires are never proved owing to their destruction or decay before their worth has become known. Generally a bull reaches the age of 5 or 6 years before even the first of his daughters comes into production, and it takes a few more years before there is a sufficient number of daughters to indicate his value as a transmitter. With co-operative artificial breeding, it has become possible to have a sufficient number of daughters to prove a bull earlier than under natural conditions. Due to the progress made in the storage and transport of semen, the mating of widely separated animals has been brought within the reach of reality.

In the operation of artificial breeding no mating involving actual contact of animals takes place, and therefore the danger of dissemination of certain contagious diseases, viz. trichomoniasis, infectious vaginitis, contagious abortion and metritis, is entirely avoided. Artificial insemination under large-scale field conditions used rationally or in conjunction with expert diagnosis by veterinary surgeons can play an important role in the control of certain well-defined reproductive disorders of livestock.

Apart from the control of venereal diseases ensured by the strict and regular examination of bulls by veterinary surgeons, sterility in cows due to anatomical deformity can be easily overcome by artificial breeding. With cows which fail to breed after repeated services, it is desirable to try artificial insemination before recommending their destruction. During extreme cold weather breeding bulls have been noticed to suffer from temporary sterility or low breeding efficiency which is said to be associated with vitamin C deficiency. This condition is often not detected until a large number of cows have been covered, and this causes a considerable disturbance of the breeding programmes. It is an advantage, therefore, to be able to collect the semen of those bulls by means of an artificial vagina and test it, as soon as the condition is suspected.

Some recent work in the U.S.S.R. indicates the possibility of artificial determination of sex by the separation of male and female-determining spores by the method of electrophoresis, i.e. accumulation of male and female-determining spores in cathode and anode respectively. The success of this method will have a striking effect on the future development of dairying and animal industry.

It was not until 1933 that the first detailed account of the progress and technique of artificial insemination was published in Great Britain by Walton, who himself is now acknowledged to be one of the leading authorities in this field.

The application of artificial insemination on organized lines in Great Britain offers great possibilities in live-stock improvement in that it provides the small farmers, who are responsible for producing one half of the total milk yield, with the use of a much better sire than they could individually own and thus the non-pedigree breeders will have the opportunity of grading up their stock. Results observed in the U.S.A. and Denmark appear to indicate that the suggestion of a possible adverse effect of artificial insemination on the trade of pedigree sires in Great Britain is without any foundation, but on the contrary it will provide an incentive to breeders who are eager to improve their important stocks for post-war periods. The contemplated legislation by the Ministry of Agriculture to safeguard the successful establishment of this practice by restraining its use to properly authorized persons in all

cases and the need for close scrutiny of breeding bulls to be used for fertility and quality indicate that the veterinary surgeon, in virtue of his training and experience, is eminently suited to supervise the work of organized artificial breeding. In Great Britain there is evidence of growing interest amongst small breeders and dairy farmers as to the necessity of organized artificial breeding. This is borne out by the fact that two schemes for the formation of artificial breeding centres at Cambridge and Reading have been approved by the Ministry of Agriculture. Similarly private schemes and those sponsored by breed societies are being launched in co-operation with the veterinary profession. [S.K.S.]

The effect of processing on the nitrogen distribution in milk. S. G. MENEFEE, O. R. OVERMANN and P. H. TRACY (1941). *J. Dairy Sci.* 11, 953-68

ROWLAND's semi-micro procedure for the determination of total casein, non-casein, non-protein, globulin, albumin and proteose nitrogen was adopted for some processed milks which gave the following results with the mixed herd milk. (1) Homogenization of skim milk, 4 per cent whole milk and 8 per cent whole milk at normal (2,500 lb.) and abnormal (3,500 and 5,000 lb.) pressures gave no significant changes in the nitrogen distribution. (2) Forewarming of milk at 203°F. before condensing coagulated all the albumin which is co-precipitated with casein at a pH 4.6, with the casein causing high results for this fraction. This change is significant in all samples of condensed milk. (3) In evaporated milk the significant changes are the coagulation of albumin and most of the globulin, and an increase in non-casein nitrogen indicating hydrolysis of casein nitrogen. (4) Condensed skim milk gave minor changes in the nitrogen distribution. (5) Holder pasteurization (145°F. for 30 minutes) of milk produced no changes in nitrogen distribution. (6) Repasteurization of skim milk at 190°F. for 30 minutes coagulated all the albumin. (7) In cultured milk slight hydrolysis of casein occurred. Pasteurized skim milk was re-pasteurized for 30 minutes at 190°F. and cooled to 70°F. and starter was added at the rate of 1 quart of starter to 25 gallons of milk. The milk was kept overnight. This cultured milk (acidity 0.79 per cent) was analysed on the next day. A decrease in casein nitrogen occurred which was attributed to slight hydrolysis. The buffers used (sodium acetate and acetic acid) in the determination of non-casein nitrogen and proteose peptone (plus non-protein nitrogen) tend to function efficiently in separating the protein fractions in the cultured milk, regardless of the high acidity. The non-casein, non-protein and proteose nitrogen fractions of these products increased in percentage of nitrogen. Skim milk, sterilized cultured and sealed in tin cans showed results comparable with cultured milk. (8) Addition of 1 part of trypsin, Emulac, and steapsin to 25,000 parts of 4 per cent raw milk kept at 145°F. for 30 minutes produced definite hydrolysis of milk proteins, especially of the casein nitrogen. (9) There is no relationship between nitrogen distribution and curd tension. The raw milk, pasteurized milk, enzyme treated milk, homogenized milk and evaporated milk gave curd tension 57, 53, 26, 12 and zero grams respectively. Tentative official and semi-micro methods for determination of casein gave higher results than the official method which is attributed to the pH of the filtrates. The semi-micro method of Rowland compares favourably with the official method for the nitrogen distribution in milk. [C. P. A.]

The interrelationship of manganese, phosphatase and vitamin D in bone development (1942).

G. F. COMBS, L. C. NORRIS and G. F. HEUSER.
J. Nutr. 23, 101.

The authors demonstrated the role of manganese in bone formation by observing the effect of manganese deficiency upon bone phosphatase activity and calcification in chicks fed on rachitogenic and non-rachitogenic diets. Two series of experiments were conducted, using Rhode Island Red chicks as the experimental animals. The results showed that the bone phosphatase activity of the chicks fed on an adequate diet, except for a lack of manganese, was markedly reduced as compared to that of chicks fed on the same diet supplemented with manganese. In rachitic chicks, it was found that the bone phosphatase activity was increased to almost twice the amount found in normal chicks. By omitting manganese from the rachitogenic diet, the bone phosphatase was decreased to approximately the normal amount. The lowering of the bone phosphatase level in manganese deficiency shows that there is an intimate relationship between the phosphatase activity of the bones and the manganese content of the diet.

The ash content of the bones of chicks fed on an adequate diet, except for a deficiency of manganese, was slightly lower than that of the bones of normal chicks. The examination of the longitudinal sections of the tibiae, after staining with silver nitrate, showed that the metaphyses of the tibiae of the chicks fed on the manganese-deficient diet were approximately one-half as wide as the metaphyses of the tibiae of the normal chicks. The epiphyses of the tibiae of the chicks fed on the manganese-deficient diet were very weakly united to the diaphyses. The results suggest that in manganese deficiency the lowering of the phosphatase level retards bone development with the result that the bones are shortened, the ash content of the bones is reduced and the strength of the union between the epiphysis and the diaphysis is greatly reduced. [S. B.]

A filterable virus, the cause of a respiratory nervous disorder of chickens. D. E. STOVER (1942). *Amer. J. Vety. Res.*, 3, 207.

RESPIRATORY nervous disorder is a serious disease affecting young growing chickens in North California, and is caused by a filterable virus. The disease affects fowls in the age range of three to ten weeks. Some

outbreaks have been observed in younger chickens and in mature hens also.

The incubation period in artificially infected chicken is found to vary between two and six days, with an average of four days. The disease runs a protracted course both in the neck and in the individual bird.

The respiratory and general symptoms consist of apnoeicness, sneezing, shaking of the head, a husky voice, gasping for breath, breathing becomes laboured as the disease progresses and the body temperature may be between subnormal and 111°F. These are followed by nervous symptoms which are of three general types. They are in co-ordination of the muscles of the neck and leg, constant tremor of the body and paralysis of the legs.

The post-mortem examination reveals a cloddiness of the air sacs in the early stages, followed by oedema. The contents of the air sacs become purulent; and adherent to the membrane are found particles of yellow caseous material. A frothy colourless serous fluid occurs in the body cavity and the froth-covered mesenteric has a cloudy or turbid appearance. A good many cases show oedema or congestion of the lungs and the presence of frothy mucus in the bronchioles and lower portion of the trachea. In a few cases bronchitis and tracheitis and the presence of a caseous material in the sinuses is noticed.

Histological and macroscopical examination of the brain and nervous system failed to detect any specific lesions.

The virus is found to be present in the air sacs, lungs and trachea of affected birds. The disease can be transmitted experimentally by inoculation with infected material intranasally, intratracheally, subcutaneously and by injecting directly into the crop. The disease can also be transmitted by pen contact. In nature, the usual method of transmission is by the ingestion of food and water contaminated with the excreta of sick fowls. A rapid passage of the infective agent through several lots of chickens resulted in a shortening of the incubation period from an average of five days to an average of three days.

Macerated saline emulsion of infective material from the air sacs, lungs and trachea were filtered through Berkefeld V. N and W filters and the filtrates inoculated in chickens developed the disease which demonstrated that the disease is caused by a filterable virus.

Cross-immunity tests which were carried out indicated that the virus of Respiratory nervous disorder is distinct from the virus of infectious bronchitis and from that of infectious laryngo-tracheitis. [P. R. K.]

ORIGINAL ARTICLES

CLIMATIC FACTORS AS RELATED TO THE INCIDENCE OF EQUINE STRANGLES IN INDIA

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(With 12 text-figures)

In a previous article [Minett, 1944] an account was given of the recorded incidence of strangles at the Army Remount Depots of Mona and Sargodha in the Shahpur District of the Punjab and at the Stud Farms of Probynabad, Coleyana and Renala in the Montgomery District of the Punjab and of Ahmednagar in Bombay. These records extend back to 1917 and 1927 in the case of Mona and Sargodha, while in the case of the stud farms they cover varying periods of years between 1914 and 1942. In that article the animal husbandry arrangements at the depots and farms were described and the problem of controlling the disease in remount depots was dealt with. In the present paper an attempt is made to throw further light on the question whether conditions of climate are in any way concerned with the prevalence of the disease at the places mentioned.

DATA

Meteorological. The following data were supplied by the India Meteorological Department:

District or station	Period	Elements
Montgomery Sargodha Ahmednagar	1914—41 1927—40 1928—41	(i) Monthly rainfall, in inches; (ii) Maximum temperature, in °F; (iii) Minimum temperature, in °F; (iv) Relative humidity in per cent, at 8 hours L.M.T.; (v) Wind velocity, in m.p.h.; (vi) Wind direction, normal percentage frequencies by months at 8 hours L.M.T. and 17 hours I.S.T.; (vii) Average number of days of dust storms, by months. [For (i) to (v) means of daily figures were given by months.]
Montgomery (District) Shahpur (District)	1918—40	District average of rainfall, by months.

DATA—contd.

District or station	Period	Elements
Sargodha Montgomery	1936—40 (a) 1890—1920 (b) 1936—40	(vi) Wind direction, normal percentage frequencies by months (a) at 8 hours L.M.T. and (b) at 17 hours I.S.T.
Sargodha Montgomery	1935—39	(vii) average number of days of dust storms, by months.

It is to be noted that Mona is 40 miles north of Sargodha and that the nearest observatory is at Sargodha, and also that Ahmednagar stud farm is quite close to the observing station at that town. Figures for daily rainfall and daily maximum and minimum temperatures were also obtained from each remount depot and from the Okara Military Farm, which is situated at a distance of 5 and 13 miles to the west of Coleyana and Renala respectively. Renala is eight miles east of Coleyana, while Probynabad is 20 miles to the south. Montgomery town, the nearest observatory to Okara, is at a point about 23 miles to the west. Monthly average rainfall figures for Renala were also available. Altitudes are as follows: Sargodha, 614' Montgomery 558'; Ahmednagar, 2154'.

The data supplied by the Meteorological Department have been averaged to give the mean monthly figures for the period during which the case incidence of strangles is being considered. The figures, with the exception of those for Wind Direction, are shown in Table I.

Strangles incidence and mortality by months. The figures for the depots and farms are set out in Table II, horses and mules being shown separately, while in Table III the total incidence figures from Table II have been arranged according to certain well-defined seasons. In Figs. 1 to 6 the meteoro-

TABLE I
Meteorological data

	January	Feb.	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Annual average for period named	Annual average for normal (%)
<i>Rainfall</i>														
(1)	0.74	0.78	0.82	0.61	0.88	1.26	3.66	3.88	1.04	0.15	0.19	0.23	13.74	
(2)	0.80	1.42	1.22	0.92	0.98	1.54	4.09	4.44	1.51	0.30	0.14	0.43	17.68	
(3)	0.71	1.14	1.16	0.43	0.30	1.69	3.62	3.88	1.15	0.05	0.09	0.11	14.34	
(4)	0.79	0.83	0.64	0.62	0.43	1.51	4.05	3.31	0.62	0.03	0.06	0.22	13.12	
(4a)	0.78	0.89	1.10	0.78	0.68	1.15	3.60	3.55	1.48	0.15	0.16	0.32	..	14.64
(5)	0.28	0.29	0.33	0.39	0.45	0.62	2.72	2.95	1.46	0.15	0.06	0.23	9.93	10.56
(5a)	0.53	0.50	0.43	0.31	0.31	0.81	2.63	2.47	1.25	0.05	0.06	0.21	..	9.55
(6)	0.43	0.59	0.40	0.28	0.20	1.12	2.98	2.15	0.65	0.07	0.06	0.18	9.11	
(7)	0.58	1.11	0.44	0.22	0.11	1.44	2.90	1.12	0.40	0.02	0.00	0.26	8.6	
(8)	0.49	1.04	0.43	0.34	0.22	1.52	2.07	2.65	0.57	0.01	0.00	0.24	9.88	
(9)	0.52	0.81	0.40	0.33	0.23	1.42	3.27	4.34	0.58	0.07	0.04	0.41	12.42	
(10)	0.01	0.03	0.04	0.49	1.46	5.72	3.82	3.67	7.38	3.74	1.72	0.43	28.51	22.33
<i>Maximum temperature</i>														
(1)	66.5	70.8	81.2	92.8	103.9	106.6	100.6	98.5	98.9	93.4	82.0	71.5		
(3)	66.6	71.8	79.7	91.1	104.5	106.1	101.1	98.0	90.0	93.7	83.1	72.6		
(5)	68.3	72.9	84.6	95.2	103.1	107.2	102.0	98.3	98.1	93.8	82.5	71.3		
(6)	67.3	72.5	83.1	94.7	105.4	106.0	100.9	99.1	99.0	94.4	83.2	72.0		
(7)	57.2	71.7	81.8	93.7	106.9	105.1	101.5	100.2	97.9	94.9	83.5	72.6		
(10)	84.2	88.6	94.9	99.6	102.3	92.7	85.5	85.1	85.3	87.1	83.8	82.1		

Minimum temperature

(1)	38.4	44.0	51.8	61.2	72.6	79.3	80.5	79.2	73.4	61.3	47.9	40.0
(3)	37.6	45.4	51.4	60.3	73.1	79.3	81.3	79.1	73.7	60.6	47.2	40.5
(5)	42.0	46.7	56.8	66.8	75.4	83.2	83.7	81.4	75.6	63.8	51.3	43.5
(6)	41.1	47.8	56.2	66.8	77.2	82.6	83.0	81.4	75.8	65.7	51.6	43.6
(7)	41.1	48.8	55.7	66.0	78.4	82.2	83.3	81.9	76.0	64.9	51.8	43.7
(10)	52.8	57.0	64.1	70.9	73.1	72.6	71.0	70.0	68.7	66.9	59.0	53.5

Relative humidity

(1)	82	81	70	54	42	51	65	72	68	61	67	81
(3)	80	82	72	56	42	53	67	72	66	59	68	82
(5)	74	68	56	45	40	47	63	70	63	55	60	73
(6)	78	75	63	54	40	48	70	72	67	58	63	75
(7)	78	79	69	58	49	59	71	71	68	59	62	78
(10)	46	46	26	20	37	60	79	79	80	63	58	54

Wind velocity

(1)	0.7	1.2	1.6	1.7	1.8	2.3	2.2	1.9	1.3	0.8	0.5	0.6
(3)	0.9	1.5	1.9	2.1	2.0	2.8	2.7	1.9	1.4	0.8	0.5	0.6
(5)	2.1	2.7	3.1	3.4	3.8	4.7	4.9	4.1	3.2	2.1	1.6	1.8
(6)	1.6	2.0	2.4	2.3	2.6	3.5	3.3	2.9	2.2	1.7	1.2	1.3
(7)	1.2	1.6	2.0	1.9	2.2	3.2	2.7	2.4	2.0	1.5	1.0	1.0
(10)	3.5	4.0	4.7	5.4	6.5	7.0	7.6	6.9	5.2	4.7	4.2	3.5

Dust storm

(1)	0.0	0.0	0.0	0.6	1.2	2.8	1.2	1.0	0.4	1.0	0.0	0.0
(5)	0.0	0.0	1.0	0.4	2.6	5.2	2.2	3.0	0.4	0.8	0.0	0.2

Data for 1935-39

(1) Sargodha, 1927-40, for Mona; (2) Mona Depot, 1922-42; (3) Sargodha, 1933-40, for Sargodha; (4) Sargodha Depot, 1927-40; (4a) Shahpur District, normal average (*); (5) Montgomery, 1914-34, for Prolvalval; (5a) Montgomery District, normal average (*); (6) Montgomery, 1928-41, for Colwyn; (7) Montgomery, 1935-41, for Renala; (8) Renala Estate, 1933-43; (9) Okara (Military Farm), 1938-42; (10) Ahmednagar, 1931-37, for Ahmednagar

(*) Based on data up to 1920

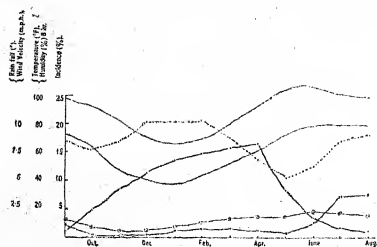


FIG. 1. Mona, 1927-40 (6097 cases)

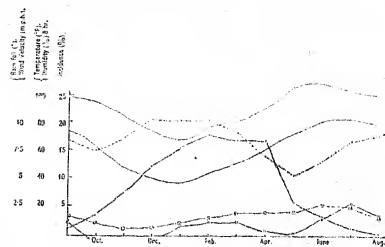


FIG. 2. Sargodha, 1933-40 (3098 cases)

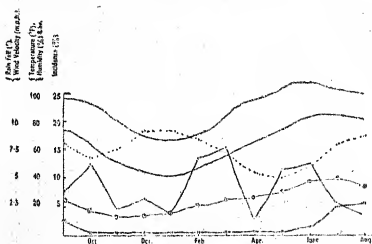


FIG. 3. Probynabad, 1914-34 (830 cases)

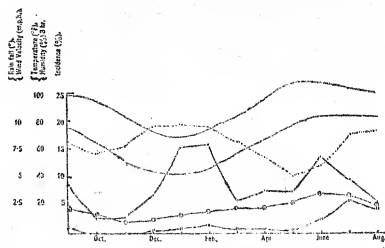


FIG. 4. Coleyana 1930-42 (427 cases) meteorological data are for 1928-41

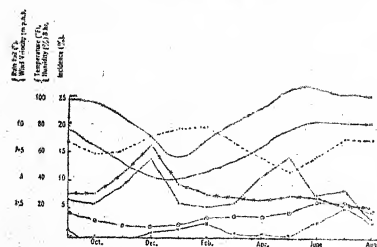


FIG. 5. Renala, 1935-41.

404 cases excluding 1938

643 cases including 1938

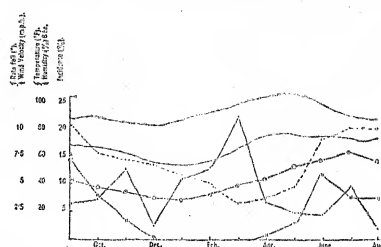
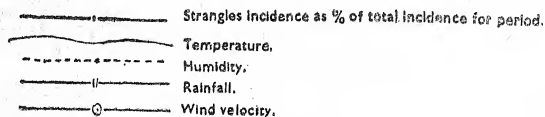


FIG. 6. Ahmednagar, 1931-37 (180 cases)



logical data from Table I (except those for 'dust storms') and the incidence figures for the corresponding period are displayed graphically. Finally in Figs. 7 to 12 local meteorological data for daily maximum and minimum temperature and rainfall are plotted, beside daily case incidence. Naturally, it would have been of value to examine figures for breeding farms situated in other provinces with a different climate, but such figures are not available.

ANALYSIS OF DATA

Examination of Figs. 1 to 6

Inspection of Figs. 1 and 2 shows that at the remount depots the attack rate rises sharply from September-October when newly purchased young stock begin to arrive and reaches a peak in April to fall abruptly during May and June, and the rate then remains low till September. On the Punjab stud farms (Figs. 3 to 5) the curve is not so simple. There is agreement, however, in that the attack rate declines in July-August. At Coleyana and Renala the curve shows a winter rise and a sharp secondary rise between April and June. At Probhynabad the rise expected in the colder months is delayed till February-March, while the secondary rise in May-June is still present. At Ahmednagar (Fig. 6), which has to be considered separately, because it is situated in an entirely different part of the country, and has a different and more equable climate, the incidence rises during the drier winter months and is much lower from April to October.

The incidence may now be examined in relation to the meteorological factors.

Rainfall. On the stud farms at least the declining incidence curve of July and August is associated with a rising rainfall. The relation to rainfall is particularly evident at Ahmednagar. Clearly it appears that monsoon conditions, even when the total rainfall is light, are not favourable to the spread of strangles.

Relative humidity (8 a.m. reading). Here there is no clear connection. As we have seen, the attack rate is low during the summer monsoon when the relative humidity is, of course, fairly high. But in the winter, when the relative humidity is still comparatively high, the disease may reach the dimensions of a steady outbreak, while cases may still be appearing as late as April-May when the humidity is low.

Temperature. In all the charts for the Punjab stations, except that for Probhynabad, there is distinct evidence that a declining air temperature is associated with an autumn rise in the number of case admissions. At the stud farms a smaller rise is noted in the pre-monsoon days of summer, when the maximum temperature is very high. Here it may be mentioned that at the depots this summer incidence rise would not

be expected because by that time the number of susceptible stock is small.

Wind velocity and direction. On the stud farms velocity *per se* appears to bear no relationship to strangles morbidity, since at the time of year, when the velocity is higher, the incidence first rises and then falls. As regards direction, the data (not included in this paper) show that at Sargodha and Montgomery the prevailing breeze is northerly (N., N.E., or N.W.) from December or January until April or May, and that during the rest of the year it is southerly (S., S.E., or S.W.). Thus, during the winter season, when air temperatures are low and especially when there is rain, the northerly breeze, though not of high velocity, is likely to be severely felt by exposed animals. During the monsoon, the southerly wind, though of higher velocity, is unlikely to have an ill-effect, since the temperature is then higher.

Dust storms. From Table I it may be seen that at the Punjab stations storms are most frequent in the period May-August and especially in June. It seems possible that the weather phenomena responsible for these storms and especially the hot and dry southerly wind at this season may be concerned with the increased number of cases on the Montgomery stud farms in May and June.

Years of high and low incidence on stud farms.

Reference to Fig. 1 of the previous paper, giving case admissions at the stud farms by years, will show that in some years the disease is very prevalent, and in others absent or nearly so. It was of interest to see if this periodicity might be ascribed to weather conditions. For this purpose, eight years were taken, in which the attack rate in the Punjab studs was 42 per cent or more of the young stock population and compared with eight years in which the rate did not exceed 2.4 per cent. For each of these 16 years, the following meteorological elements were examined: rainfall for the year, rainfalls for February-June, July-September and October-January, and the mean relative humidity and mean maximum temperature for these three seasons. One might perhaps have anticipated that, during the years when there was little or no disease, the rainfall figures at least might have been above the average, but no variation of this sort was found. It seems, therefore, that the variation in incidence from one year to another on the stud farms must be attributed to some other factor or factors than climate. There is no indication that the variation is due to a differing density in the population of young horses. No doubt, the simple explanation is that a bad outbreak in a particular year leaves most of the population immune, since years of high incidence at Probhynabad and Coleyana are usually succeeded by three or four years in which the incidence is low.

TABLE II
Incidence of strangles by months

Year	Months												Total
	Septem-ber	October	Novem-ber	Decem-ber	January	February	March	April	May	June	July	August	
MONA (HORSES)													
1926-27 . . . { (1) (2)	0 (0-0)	15 (4-4)	33 (0-7)	35 (10-3)	37 (10-8)	74 (21-7)	17 (5-0)	44 (18-0)	49 (14-4)	25 (7-4)	4 (1-2)	7 (2-1)	340
1927-28 . . . { (1) (2)	11 (3-2)	26 (7-8)	41 (12-4)	53 (16-0)	51 (15-4)	29 (6-0)	17 (5-1)	70 (21-1)	20 (6-0)	12 (3-9)	10 (3-0)	0 (0-0)	332
1928-29 . . . { (1) (2)	1 (0-2)	3 (0-8)	5 (1-4)	38 (10-9)	70 (20-0)	91 (26-0)	29 (5-7)	57 (16-3)	37 (10-6)	15 (4-3)	10 (2-9)	3 (0-8)	350
1929-30 . . . { (1) (2)	11 (3-4)	12 (3-6)	9 (2-8)	12 (3-6)	40 (12-2)	44 (13-5)	60 (18-4)	69 (21-1)	28 (8-0)	12 (3-6)	17 (5-2)	15 (4-0)	327
1930-31 . . . { (1) (2)	16 (8-2)	20 (4-0)	80 (15-9)	67 (13-4)	77 (15-3)	43 (8-5)	33 (6-6)	60 (13-8)	39 (7-7)	37 (7-4)	14 (2-8)	7 (1-4)	502
1931-32 . . . { (1) (2)	8 (1-9)	67 (15-8)	21 (5-0)	36 (8-3)	40 (9-4)	38 (9-1)	92 (21-7)	65 (15-3)	21 (5-0)	8 (1-9)	14 (3-3)	0 (0-0)	424
1932-33 . . . { (1) (2)	10 (1-8)	35 (0-2)	89 (15-6)	27 (4-7)	80 (14-0)	72 (12-6)	93 (16-3)	68 (11-9)	44 (7-7)	35 (6-2)	13 (2-3)	4 (0-7)	370
1933-34 . . . { (1) (2)	7 (1-2)	5 (0-9)	30 (5-2)	29 (5-1)	47 (8-2)	121 (21-0)	117 (20-3)	120 (20-5)	60 (10-4)	32 (5-6)	6 (1-1)	1 (0-2)	575
1934-35 . . . { (1) (2)	0 (0-0)	3 (0-5)	39 (7-0)	52 (9-3)	80 (14-4)	89 (16-0)	153 (27-5)	40 (10-2)	33 (5-9)	10 (1-8)	5 (0-9)	3 (0-5)	557
1935-36 . . . { (1) (2)	0 (0-0)	19 (3-2)	53 (9-7)	91 (15-2)	113 (18-9)	56 (9-4)	68 (11-4)	35 (13-5)	73 (12-2)	14 (2-4)	7 (1-1)	6 (1-0)	568
1936-37 . . . { (1) (2)	2 (0-3)	6 (1-0)	1 (0-2)	26 (4-4)	111 (13-0)	171 (23-0)	124 (21-2)	98 (10-3)	43 (7-2)	8 (1-4)	2 (0-3)	2 (0-3)	596
1937-38 . . . { (1) (2)	3 (0-6)	24 (4-4)	58 (10-3)	180 (33-1)	56 (10-3)	55 (10-1)	70 (14-5)	49 (9-0)	29 (5-3)	2 (0-4)	5 (0-9)	4 (0-7)	544
1938-39 . . . { (1) (2)	4 (1-2)	23 (7-1)	13 (4-0)	7 (2-2)	26 (8-0)	67 (20-6)	60 (18-4)	57 (20-3)	33 (10-2)	3 (0-9)	1 (0-3)	1 (0-3)	325

1830-40 . . .	{ (1) (2)	1 (0-3) 0	43 (12-1) 0	55 (13-9) 1	73 (18-4) 2	32 (5-5) 3	22 (5-3) 2	54 (13-6) 0	64 (10-1) 3	40 (11-5) 4	9 (2-3) 3	3 (0-3) 0	0 (0-0) 0	397*
Totals 1929-40 . . .	{ (1) (2)	74 (1-2) 0	306 (4-8) 3	532 (8-3) 14	726 (11-3) 28	850 (13-2) 24	968 (15-0) 29	989 (15-3) 54	1043 (16-2) 75	553 (8-5) 42	223 (3-5) 24	111 (1-7) 4	67 (1-0) 1	6437
Number of deaths expressed as the percentage of total number of births		(0-0)	(1-0)	(4-7)	(9-4)	(3-1)	(9-7)	(18-1)	(25-2)	(14-1)	(8-1)	(1-3)	(0-3)	...

SARGODHA (HORSES)

1933-34 . . .	{ (1) (2)	8 (2-4) 1	2 (0-6) 0	1 (0-3) 0	2 (0-6) 0	40 (11-9) 1	43 (12-8) 2	49 (14-5) 3	66 (10-5) 4	39 (10-7) 5	62 (18-4) 4	21 (6-2) 5	7 (2-1) 0	337
1934-35 . . .	{ (1) (2)	11 (2-1) 0	47 (8-7) 2	56 (10-5) 2	48 (9-0) 1	116 (21-7) 4	82 (15-3) 2	41 (7-7) 1	75 (14-0) 4	32 (6-0) 5	18 (3-3) 0	7 (1-3) 0	2 (0-4) 0	535
1935-36 . . .	{ (1) (2)	4 (0-8) 0	19 (4-0) 0	71 (14-5) 2	94 (19-5) 1	55 (11-4) 5	78 (10-2) 0	76 (15-8) 3	63 (13-1) 4	10 (2-1) 2	9 (1-9) 0	2 (0-4) 0	0 (0-0) 0	481
1936-37 . . .	{ (1) (2)	1 (0-2) 0	8 (1-6) 0	6 (1-3) 0	100 (22-2) 0	97 (21-7) 14	95 (21-2) 5	40 (8-9) 4	72 (16-0) 8	25 (5-6) 0	2 (0-4) 0	4 (0-0) 0	0 (0-0) 0	450
1937-38 . . .	{ (1) (2)	0 (0-0) 0	20 (4-3) 1	77 (16-3) 1	85 (18-2) 3	25 (5-4) 1	38 (8-1) 0	74 (15-8) 4	103 (22-1) 3	37 (7-0) 15	4 (0-9) 2	2 (0-4) 0	2 (0-4) 0	407
1938-39 . . .	{ (1) (2)	3 (0-9) 0	6 (1-8) 0	2 (0-6) 0	16 (4-7) 0	56 (16-6) 2	63 (18-0) 5	103 (30-5) 10	65 (19-2) 6	17 (5-0) 8	7 (2-1) 0	0 (0-0) 0	0 (0-0) 0	338
1939-40 . . .	{ (1) (2)	0 (0-0) 0	0 (0-0) 0	9 (1-5) 0	29 (5-3) 0	88 (18-0) 2	147 (30-0) 7	128 (26-1) 14	66 (13-5) 2	28 (5-3) 1	0 (0-0) 0	0 (0-0) 0	0 (0-0) 0	400
Totals 1933-40 . . .	{ (1) (2)	27 (0-9) 1	102 (2-3) 3	222 (7-2) 5	371 (12-0) 5	477 (15-4) 20	546 (17-0) 24	611 (16-4) 39	510 (16-4) 31	185 (5-9) 42	102 (3-3) 6	36 (1-2) 5	11 (0-4) 0	3098
Number of deaths expressed as the percentage of total number of deaths		(0-0)	(1-6)	(2-6)	(2-6)	(15-3)	(12-6)	(20-5)	(16-3)	(22-1)	(3-2)	(2-6)	(1-0)	...

* Figure '397' includes 7 animals readmitted for strangles and 7 cases among stud stock

TABLE II—contd.

Year	Months												Total
	Septem- ber	October	Novem- ber	Decem- ber	January	February	March	April	May	June	July	August	

MONA (MULES)													
1933-34	{ (1) (2)	1 (0-5) 0	2 (0-9) 0	3 (1-4) 0	23 (10-8) 0	40 (18-7) 0	29 (13-5) 1	84 (39-2) 0	23 (10-8) 1	6 (2-8) 0	2 (0-9) 0	0 (0-0) 0	214
1934-35	{ (1) (2)	0 (0-0) 0	3 (1-3) 0	56 (24-3) 0	20 (12-8) 0	10 (4-4) 0	51 (22-6) 0	62 (27-4) 1	8 (3-5) 0	6 (2-7) 0	1 (0-4) 0	0 (0-0) 0	226
1935-36	{ (1) (2)	0 (0-0) 0	7 (2-1) 0	88 (28-4) 0	45 (13-3) 0	4 (1-2) 0	59 (17-7) 2	111 (33-3) 0	15 (4-0) 0	4 (1-2) 0	0 (0-0) 0	0 (0-0) 0	383
1936-37	{ (1) (2)	0 (0-0) 0	0 (0-0) 0	2 (0-5) 0	85 (22-4) 0	188 (30-3) 0	33 (8-7) 1	113 (31-0) 2	2 (0-5) 0	1 (0-3) 0	1 (0-3) 0	0 (0-0) 0	350
1937-38	{ (1) (2)	2 (0-6) 0	0 (0-0) 0	69 (16-0) 0	43 (11-9) 0	35 (9-6) 0	38 (10-5) 0	24 (6-5) 0	5 (1-3) 0	2 (0-0) 0	1 (0-3) 0	0 (0-0) 0	362
1938-39	{ (1) (2)	1 (0-4) 0	1 (0-4) 0	21 (8-3) 0	60 (24-7) 0	34 (12-2) 0	114 (40-5) 0	34 (12-1) 1	1 (0-4) 0	1 (0-4) 0	0 (0-0) 0	0 (0-0) 0	279
1939-40	{ (1) (2)	1 (0-3) 0	41 (12-0) 0	24 (7-0) 0	44 (12-8) 0	96 (27-0) 2	113 (32-0) 4	12 (3-5) 1	5 (1-5) 0	4 (1-2) 0	1 (0-3) 0	0 (0-0) 0	343
Totals 1933-40	{ (1) (2)	5 (0-2) 0	113 (3-3) 0	145 (16-2) 1	295 (13-8) 4	305 (14-3) 1	420 (19-7) 5	346 (25-5) 8	66 (3-1) 4	25 (1-2) 0	9 (0-4) 0	2 (0-1) 0	2137
Number of deaths expressed as percentage of total number of mules	{ (1) (2)	(0-0) 0	(0-0) 0	(4-4) 0	(17-4) 0	(4-4) 0	(21-7) 0	(34-7) 0	(17-4) 0	(0-0) 0	(0-0) 0	(0-0) 0	..

SARGODHA (MULES)													
1933-34	{ (1) (2)	0 (0-0) 0	1 (0-6) 0	0 (0-0) 0	20 (11-4) 1	36 (20-7) 0	19 (46-4) 0	32 (18-4) 1	4 (2-3) 0	2 (1-2) 0	0 (0-0) 0	0 (0-0) 0	174
1934-35	{ (1) (2)	0 (0-0) 0	4 (2-3) 0	8 (3-0) 0	41 (25-3) 0	22 (12-0) 1	44 (25-3) 0	46 (26-4) 0	3 (1-7) 0	1 (0-6) 0	0 (0-0) 0	0 (0-0) 0	174
1935-36	{ (1) (2)	0 (0-0) 0	0 (0-0) 0	2 (0-8) 0	1 (0-4) 0	37 (23-6) 0	114 (47-1) 0	60 (24-8) 2	5 (2-1) 0	1 (0-4) 0	0 (0-0) 0	2 (0-5) 0	242
1936-37	{ (1) (2)	2 (0-7) 0	1 (0-3) 0	55 (15-8) 0	108 (55-1) 0	38 (12-3) 0	33 (10-7) 1	50 (19-2) 1	6 (1-0) 0	2 (0-7) 0	0 (0-0) 0	0 (0-0) 0	598
1937-38	{ (1) (2)	0 (0-0) 0	4 (0-9) 0	20 (6-3) 0	13 (4-1) 0	79 (24-9) 0	69 (21-9) 0	76 (24-0) 0	3 (0-0) 0	2 (0-6) 0	0 (0-0) 0	0 (0-0) 0	317
1938-39	{ (1) (2)	0 (0-0) 0	5 (1-5) 0	0 (0-0) 0	82 (29-6) 0	45 (22-4) 0	20 (7-2) 1	85 (30-7) 1	22 (7-0) 1	1 (0-4) 0	0 (0-0) 0	0 (0-0) 0	277

	(1)	1 (0-2)	0 (0-0)	13 (4-1)	45 (14-2)	122 (45-9)	68 (21-7)	24 (7-6)	39 (12-4)	2 (0-7)	0 (0-0)	0 (0-0)	0 (0-0)	314
1884-40	{ (2)	0	0	0	0	0	0	1	0	0	0	0	0	1
	{ (1)	3 (0-2)	8 (0-4)	74 (4-1)	133 (7-4)	300 (21-6)	302 (20-0)	385 (21-2)	237 (22-0)	45 (2-5)	9 (0-5)	9 (0-0)	2 (0-7)	1806
Totals 1882-40	{ (2)	0	0	0	0	2	4	5	5	1	0	0	0	17
Number of deaths expressed as the percentage of total number of deaths	{ (0-0)	(0-0)	(0-0)	(0-0)	(0-0)	(11-5)	(23-5)	(29-4)	(29-4)	(8-9)	(0-0)	(0-0)	(0-0)	..

PROBYNABAD (HORSES)*

	(1)	14 (11-0)	8 (0-3)	3 (2-4)	75 (30-0)	23 (18-1)	...	2 (1-6)	2 (1-6)	127
1914	{ (2)	1	1	1	1	4
1915	{ (1)
	{ (2)
1916	{ (1)
	{ (2)
1917	{ (1)	7 (100-0)	7
	{ (2)
1918	{ (1)	10 (8-2)	20 (16-5)	3 (2-5)	3 (2-5)	...	3 (2-5)	54 (44-6)	2 (1-7)	15 (12-4)	2 (1-7)	10 (8-2)	121
	{ (2)	1	2	3
1919	{ (1)	1 (20-0)	2 (40-0)	5
	{ (2)
1920	{ (1)	1 (25-0)	1 (25-0)	1 (25-0)	4
	{ (2)	...	1 (25-0)	1	1
1921	{ (1)	1 (9-1)	...	1 (9-1)	8 (72-7)	1 (9-1)	11
	{ (2)
1922	{ (1)
	{ (2)
1923	{ (1)	1 (2-0)	44 (86-3)	6 (11-7)	...	51
	{ (2)
1924	{ (1)	...	3 (4-0)	38 (51-4)	33 (44-6)	74
	{ (2)	5	1	6
1925	{ (1)
	{ (2)
1926	{ (1)	4 (7-4)	9 (16-7)	4 (7-4)	2 (3-7)	2 (3-7)	...	1 (1-9)	26 (45-1)	6 (11-1)	...	54
	{ (2)	...	2	2
1927	{ (1)	3 (7-0)	3 (7-0)	7 (16-3)	6 (14-0)	4 (9-3)	13 (30-2)	5 (11-6)	1 (2-3)	...	1 (2-3)	...	43
	{ (2)	1	1	1
1928	{ (1)	11 (14-3)	40 (51-9)	4 (5-2)	4 (5-2)	6 (7-8)	10 (18-0)	2 (2-0)	...	77
	{ (2)	3	1	2	2	1	...	9

* Includes both young and older horses since age at admission unknown

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TABLE II—contd.

Year	Months												Total
	Septem-ber	October	Novem-ber	Decem-ber	January	February	March	April	May	June	July	August	
PROBYNABAD (HORSES)*—contd.													
1929	{ (1) (2)	9 (25-7) 1	4 (11-4) 1	3 (8-6) ...	2 (5-7) 1	...	13 (37-2) ...	4 (11-4) 1	35 4
1930	{ (1) (2)	11 (39-2) 2	...	1 (3-5) ...	9 (17-8) 1	2 (7-2) 1	2 (7-2) 2	2 (7-2) 1	2 (7-2) ...	3 (10-7) 1	28 8
1931	{ (1) (2)	4 (7-0) 1	11 (21-5) 1	8 (15-7)	2 (3-9) 2	7 (13-7) 1	...	15 (29-4) 1	51 5
1932	{ (1) (2)	5 (8-2) ...	1 (1-6) ...	7 (11-5) ...	13 (21-4) 1	1 (1-6) ...	6 (9-8) ...	5 (8-2) ...	5 (8-2) ...	16 (26-2) 1	...	2 (3-3) ...	61 2
1933	{ (1) (2)	1 (1-5) ...	1 (1-5) ...	3 (4-3) ...	15 (21-6)	16 (23-2) ...	9 (13-0) 1	6 (8-7) ...	1 (1-5) ...	1 (1-5) ...	69 1
1934	{ (1) (2)	7 (58-3) ...	5 (41-7)	12 ...
Totals 1914-34	{ (1) (2)	63 (7-0) 7	106 (12-8) 8	40 (4-8) 3	52 (6-3) 4	...	113 (13-6) 4	127 (15-3) 4	24 (2-9) 7	93 (11-2) 3	47 (5-6) ...	26 (3-1) 2	880 47
Number of deaths expressed as percentage of total number of deaths.		(14-9)	(16-9)	(8-5)	(8-5)	(8-5)	(8-5)	(4-3)	(14-9)	(6-4)	...	(4-3)	...
COLEYANA (HORSES)													
1930	{ (1) (2)	8 (10-7) ...	1 (1-3) ...	3 (4-0) ...	2 (2-7)	10 (13-3) ...	11 (14-7) 1	22 (29-3) ...	8 (10-7) ...	10 (13-3) ...	75 1
1931	{ (1) (2)	4 (50-0) 1	3 (37-5)	1 (12-5)	8 1
1932	{ (1) (2)	...	2 (5-4)	7 (18-9) 1	14 (37-8) 3	6 (16-3)	5 (13-5) ...	37 4
1933	{ (1) (2)	...	2 (13-3) ...	4 (26-7) ...	3 (20-0)	3 (20-0) ...	3 (20-0)	15 ...
1934	{ (1) (2)	4 (7-3) ...	2 (3-6)	14 (25-5) ...	2 (3-6) 1	17 (30-9) 4	3 (5-6) ...	2 (3-6) ...	11 (20-0) 2	55 8
1935	{ (1) (2)	10 (9-3) 1	5 (4-6) ...	29 (26-9) 3	2 (1-8) 1	...	9 (8-3) 1	12 (11-1) ...	7 (6-5) ...	3 (2-8) ...	108 7

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)
1936	20
1937	7 (8-9)	1 (1-3)	5 (6-3)	2 (2-5)	1 (1-3)	1 (1-3)	3 (2-5-3)	1 (7-7)	3
1938	3 (25-1)	79
1939	1
1940	13
1941
1942
Totals 1930-42	37 (8-7)	12 (2-8)	12 (2-8)	29 (6-8)	64 (15-0)	67 (15-7)	24 (5-6)	32 (7-4)	31 (7-3)	57 (13-3)	40 (9-4)	22 (5-2)	427							25
Number of deaths expressed as the percentage of total number of deaths.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	..

RENAL (HORSES)†

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)
1935	8 (10-4)	11 (14-3)	9 (11-7)	10 (13-0)	8 (10-3)	10 (13-0)	2 (2-0)	71 (9-1)	5 (6-3)	1 (1-3)	2 (2-0)	4 (5-2)	77							3
1936	2 (9-5)	2 (9-5)	2 (9-5)	..	2 (9-5)	1 (4-8)	2 (9-5)	1 (4-8)	3 (14-3)	3 (14-3)	1 (4-5)	2 (9-5)	21							..
1937	5 (10-2)	1 (3-9)	2 (7-7)	5 (10-2)	3 (11-5)	2 (7-7)	1 (3-9)	2 (7-7)	2 (7-7)	1							1
1938	13 (5-4)	7 (2-9)	14 (5-9)	25 (10-5)	1 (0-4)	2 (0-8)	11 (4-6)	48 (20-1)	64 (26-3)	20 (8-4)	32 (13-4)	2 (0-8)	220							..
1939	5 (5-3)	10 (10-5)	7 (7-4)	10 (10-5)	2	4	7	3	3	1	23							..
1940	11 (10-3)	7 (6-5)	22 (20-6)	7 (6-6)	1 (0-9)	4 (3-7)	19 (20-0)	6 (6-3)	4 (4-2)	4 (4-2)	5 (5-3)	5 (5-3)	95							4
1941	3 (2-8)	7 (6-5)	12 (11-2)	10 (9-4)	15 (14-0)	8 (7-5)	107							..
1942	10							..
1943	78							..
1944	3							..
1945	43							..

* Includes both young and older horses since age at admission unknown
† Excludes animals over 2 years old

TABLE II—*contd.*

Year	Months												Total
	Septem- ber	October	Novem- ber	Decem- ber	January	February	March	April	May	June	July	August	
RENALA (HORSES)*— <i>contd.</i>													
(1)	47 (6-9)	42 (6-1)	64 (9-2)	93 (13-6)	50 (7-3)	42 (6-1)	41 (6-0)	75 (10-9)	99 (14-4)	53 (7-7)	56 (8-2)	24 (3-5)	636
(2)	4	..	2	2	2	..	4	4	9	7	8	2	44
Totals 1935-42	(1**)	34 (7-6)	35 (7-6)	63 (12-2)	49 (11-0)	40 (8-9)	30 (6-7)	27 (6-0)	35 (7-8)	33 (7-5)	24 (5-4)	22 (4-9)	447
(2**)	3	..	2	1	2	..	2	..	2	4	5	1	27
Number of deaths expressed as the percentage of total number of deaths	(9-1) (0-5)	..	(4-6) (9-5)	(4-6) (4-8)	(4-6) (9-5)	..	(9-1) (9-5)	(0-1) ..	(20-4) (9-5)	(15-8) (19-1)	(18-1) (23-8)	(4-6) (4-8)	..
AHMEDNAGAR (HORSES)													
1931	(1)	1 (4-8)	2 (9-5)	..	2 (9-4)	..	3 (14-3)	..	1 (4-8)	1 (4-8)	8 (38-1)	1 (4-8)	21
(2)
1932	(1)	4 (8-0)	10 (20-0)	5 (10-0)	..	3 (6-0)	..	1 (2-0)	1 (2-0)	11 (22-0)	3 (6-0)	2 (4-0)	50
(2)	..	1	1	..	1	2	6
1933	(1)	11 (50-0)	3 (13-0)	4 (13-2)	1 (4-0)	22
(2)	..	1	1
1934	(1)	1 (1-9)	21 (40-4)	2 (3-9)	5 (9-0)	4 (7-7)	10 (19-2)	1 (1-0)	3 (5-8)	1 (1-0)	3 (5-8)	1 (1-0)	52
(2)	1	1	1	1	2	5
1935	(1)	2 (6-9)	7 (24-1)	1 (3-5)	1 (3-5)	1 (3-5)	4 (13-7)	..	5 (17-2)	..	8 (27-6)	..	29
(2)	1	1
1936	(1)	..	2 (66-7)	1 (33-3)	3
(2)
1937	(1)	1 (33-3)	1 (33-3)	..	1 (33-3)	..	3
(2)
Totals 1931-37	(1)	19 (10-6)	23 (12-7)	39 (21-7)	12 (6-7)	8 (4-5)	17 (9-4)	3 (1-7)	11 (6-1)	13 (7-2)	23 (12-7)	4 (2-2)	180
(2)	1	2	1	1	2	1	3	2	18
Number of deaths expressed as the percentage of total number of deaths	(7-7)	(15-4)	(7-7)	(7-7)	(15-4)	(7-7)	(22-0)	(15-4)

* Excludes animals over 3 years old.
 ** Excludes animals over 2 years old.
 Column (1) Number of cases admitted.
 Column (2) Number died.
 Figure bracketed indicate percentage.

Seven of the 23 animals dying in 1933 were 2 years old.

TABLE III
Case incidence of strangles in horses at depots and stud farms by months

Month	Remount Depots			Punjab—Montgomery				Bombay—Ahmednagar	
	Mina	Sargodha	Total	Probynabad	Coleyana	Renala*	Total	Month	Ahmednagar
July	111	36		47	40	24		June	8
August	67	11	326 (4.7)	26	25	22	316 (24.8)	July	17
September	74	27		63	37	34		August	3
								September	62 (34.9)
								October	11
December	726	371		52	29	68			13
January	850	477	3,932 (57.3)	33	64	49	515 (40.6)		
February	903	546		113	67	40		December	4
								January	19
April	1,043	501		24	32	27		February	23
May	553	183	2,905 (53.0)	93	31	35	438 (34.6)	March	39
June	223	102		106	57	33		April	12
			6,864				1,268		149

*Excluding 1938 which was a phenomenal year.
 Figures in brackets indicate percentages.

Examination of Tables II and III

The data have been analysed in two ways :

A. Omitting October, November and March in the Punjab and May and November at Ahmednagar as being months when climatic characters are likely to be less fixed, the year was divided into seasons and the incidence figures for these months totalled as shown in Table III. The Punjab seasons taken were (i) July to September, a moderately wet warm period ; (ii) December to February, the cold season ; (iii) April to June, the hot dry season. At Ahmednagar, (i) June to October, months of high rainfall and humidity ; (ii) December to April, a dry period with a distinctly lower mean minimum temperature than June to October.

Table III shows, firstly, that in the Punjab the attack rate is invariably lower from July to September than at any other time, though at Mona and Sargodha, as stated, this may not mean much. Secondly, the difference between the percentage figures, 57.3 and 38.0, for Mona and Sargodha for the periods December—February and April—June respectively is statistically significant. Similarly, in the totals for the stud farms there is a significant difference between the percentages, 24.8 and 34.6, 34.6 and 40.6, and 34.9 and 65.1, respectively. The suggestion, therefore, is that strangles is mainly a disease of colder months.

B. Mr. Suprakas Sen has examined the monthly attack rate from another angle. For each depot and stud farm, the attack rates of the different months, calculated from the total number of cases admitted during the period covered, have been examined and the months, in which the percentages of affected animals do not differ significantly from the highest one, have been grouped. The result of this approximate method of analysis is summarized thus :

Periods of maximum prevalence of strangles

Mona	Horses	Feb.—April
	Mules	April
Sargodha	Horses	Feb.—April
	Mules	Jan.—April
Probynabad	Feb.—Mar., June, and Oct.	
Coleyana	Jan.—Feb., June	
Renala	Dec., April, May	
Ahmednagar	March	

An analysis of mortality rates by a similar method shows that strangles mortality was prominent at,

- Mona in horses, February—March
- „ „ mules (*), January, March—May
- Sargodha in horses, January, March—May
- „ „ mules (*), January—May
- Coleyana (*), January—March, May, June

[(*) Calculations made from very small numbers and hence only approximate]

Where no mention is made, the mortality rate at different parts of the year do not differ.

Hence, from this method of analysis also it seems that the period, February to April, is most prominent for the development of strangles.

Two points are to be mentioned in connection with Tables II and III. (1) At Renala, 1938 was a phenomenal year in which 262 cases of strangles were recorded, in fact nearly all the young stock. Of these, 52 cases occurred in April and 68 in May. There is no suggestion that weather conditions were responsible, and the Estate Manager has no explanation to advance. (2) At Probynabad, high figures for strangles cases are recorded in 1924 (May and June, 71), 1923 (June, 44) and 1932 (June, 18). It is interesting that May 1924 had the highest rainfall recorded at Montgomery station in 29 years, viz. 1.62" or nearly five times the average, the next highest being May 1923 with 1.29". May 1924 also showed the lowest average maximum temperature in the 29 years, viz. 94.7°F. (average 104.0°F.), while the minimum for this month was also low, viz. 71.5°F. (average, 76.2°F.).

A further point to be noted concerns Coleyana. In 1943 strangles was prevalent at a rather unusual time of the year ; between May and September there were 76 cases of which 20 occurred in July, 30 in August and 11 in September. Most affected animals were under two years but a few were four to six years old. No weather conditions could be held responsible, and in fact, it has been noted that, with a high incidence at Coleyana, the attack rate at Renala nearby might be low, and vice versa. Probably, the main contributing circumstance of the 1943 deviation was the low incidence over several previous years.

Examination of local meteorological data

The meteorological data so far dealt with have been monthly average figures recorded at stations some miles from the depots and farms. While these suffice to show the climatic trend of the areas, they may fail to bring out the full relationship of weather conditions to the disease. In an attempt to study the matter more closely, daily records of rainfall and air temperature were obtained from the depots themselves and from a local recording station close to Coleyana and Renala. From these records, charts were prepared, on which were also inserted, where available, the daily admissions of strangles cases. In order to economize space, only selected portions of these charts are reproduced. The outbreaks in Figs. 7 (Mona) and 8 (Sargodha) began in December and were over in May. Both appear to show an association between low minimum temperature and the onset of outbreaks. It should be noted, however, that in outbreaks starting earlier in the autumn this association may not be observed.

In order to test this point further, correlation coefficients for case admissions and minimum air temperature were worked out. The coefficients proved to be negative and significant for Mona and Sargodha, negative but not significant for Coleyana. (They were: Mona —0.128*,

* The coefficients for Mona and Sargodha, though significant and therefore suggestive, are small and should not be relied upon

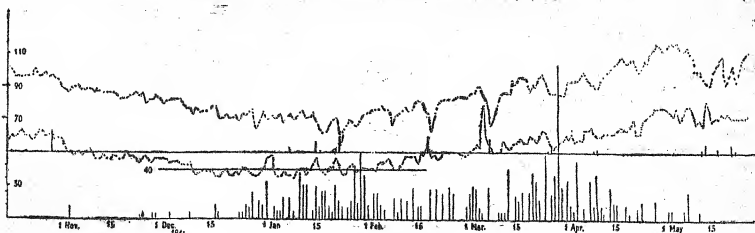


FIG. 7. Mona, 1940-41

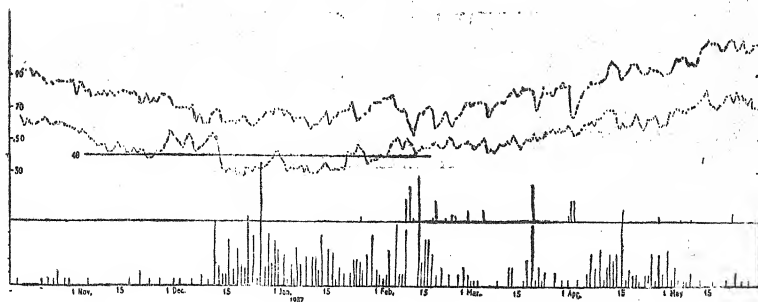


FIG. 8. Sargodha, 1936-37

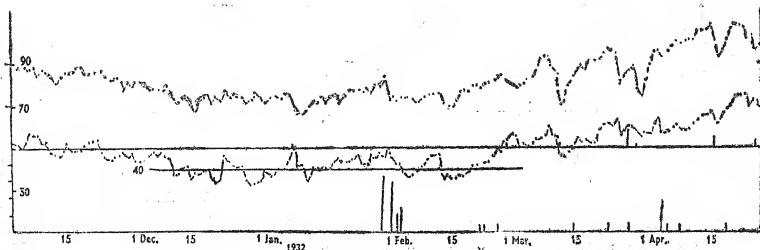


FIG. 9. Coleyana, 1931-32

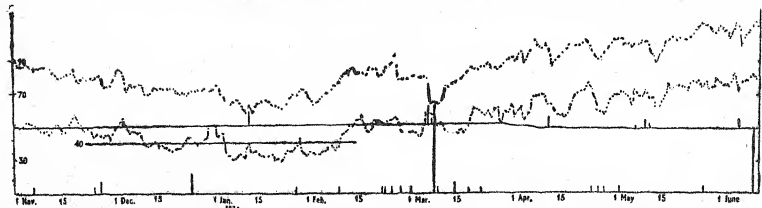
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FIG. 10. Coleyana, 1933-34

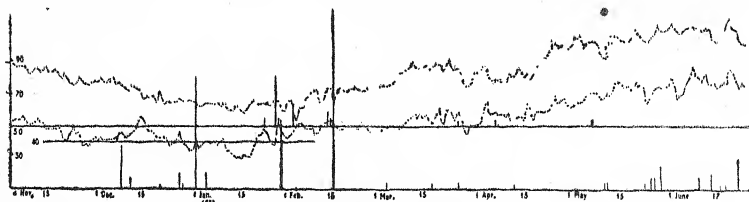


FIG. 11. Coleyana, 1934-35

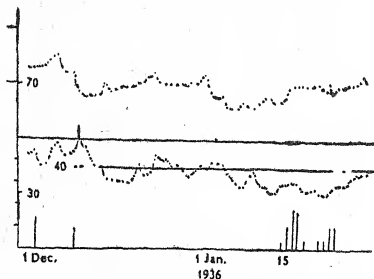
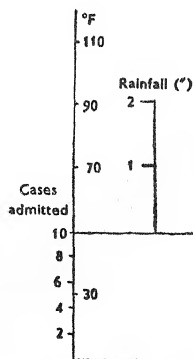


FIG. 12. Coleyana, 1935-36



Plan for Figs. 7-12

Sargodha—0.157* Coleyana—0.059, and were based on 263, 282 and 203 pairs of observations respectively. Each paired observation consisted of the

number of cases admitted on the particular day in relation to the minimum temperature noted on that day).

Since the position in respect of Coleyana (Figs. 9 to 12) is not so clear, it may be helpful to examine the figs. in the light of the following remarks.

Fig. 9. Prior to the sharp outbreak beginning on January 31 with 18 cases in five days the minimum temperature had ranged from 34 to 48°F. During the following monsoon there were only five cases. (This and other figures show clearly the sharp fall in air temperature associated with rain.)

Fig. 10. There were 15 cases between October and February. On March 6 to 8 there was a rainfall totalling 1 inch, accompanied by a sharp fall in temperature; between March 8 to 14, sixteen cases of strangles were admitted. On June 7 there was 0.3 inch rain, and three days later 10 cases of strangles were admitted. During the following monsoon, only one case appeared.

Fig. 11. Cases first appeared on December 9, there being 14 cases in that month. In January and February there were 61 cases, of which 29 were admitted in one day, February 16. During December to February minimum temperatures were very low actually below freezing point from January 14 to 18, followed during the next fortnight by heavy rainfall. Between the following May 25 and June 24 there were 21 cases, and during this period the minimum temperature fell on several occasions by 14 to 16°F. within a space of two or three days. A few cases only occurred during the following monsoon.

Fig. 12. Five cases appeared in December and 18 cases within 11 days in January, after which only three cases developed during the rest of the year 1936. The January cases were apparently associated with minimum temperatures around freezing point.

DISCUSSION

It will be useful to refer to the position in respect of diseases of other animals and of man which are acquired by the respiratory tract. In Britain and the United States, *diphtheria* is reported to reach its greatest intensity in the late autumn and winter, *scarlet fever* in the late autumn, winter and spring. *Acute rheumatism*, which is probably streptococcal in origin, has its maximum in winter or spring and is lowest in summer in temperate countries. Greenwood and Thompson [1907] found evidence of a connection between prevalence and dry weather, but no significant correlation with mean air temperature in any month. In Britain and the United States, *influenza* is highest but declining during the first half of the year, while *measles* is at its maxi-

mum in March or April. *Small-pox* in India has received much attention from the meteorological point of view by Rogers [1926] and Russell and Sundararajan [1928-9]. In most areas this disease has a tendency to greater prevalence during the first half of the year, ordinarily reaching its maximum before the rains and beginning to diminish after their commencement, and the correlation coefficients are in fact negative and significant both with rainfall and relative humidity. Some of the largest small-pox epidemics occur in the low rainfall areas of North-West and Central India and of the Deccan plateau and are least severe in Bengal with its consistently high rainfall. In the dry areas mentioned, epidemics nearly always follow a greater or less failure of the south-west monsoon, accompanied by a comparatively low humidity. The explanation presumably is that the survival of the virus is favoured by dry air [Loosli *et al.*, 1943]. It is well-known that the common cold and pneumonia are most frequent during the winter months, the meteorological factor directly or indirectly responsible for this in adults being the prevailing low temperature. In the case of pneumococcus pneumonia of man in India, Rogers [1925] has shown that its incidence is greater in the north-western part of the country, which has as its main climatic characteristics a great diurnal range of temperature (about 30°F.), a low minimum (35°F. or less) in the cold season and a low humidity. From the seasonal point of view, pneumonia is most prevalent during the dry cold season (November to February) and next during the dry-hot season (March to June). One cannot fail to note the similarity in this respect between pneumonia in man and strangles in horses. The case is said to be different with *pulmonary tuberculosis* in man, which Rogers [1925, 1] has shown is associated in Indian jails with high rainfall and high humidity and also with the direction, steadiness and strength of the rain-bearing winds. The disease is, therefore, especially prevalent in Bengal and the coastal areas of Orissa, Madras and Bombay.

In the case of animals, it may be noted that 'snuffles'—a respiratory affection of rabbits due to a *Pasteurella*—shows a seasonal periodicity, being most prevalent (in New York State) in spring, with a secondary rise in the autumn and a falling off in the summer [Webster, 1927]. Wide diurnal fluctuations in temperature raised the incidence [Webster & Burn, 1927]. The seasonal occurrence of diseases of domesticated animals in India will be taken up separately, but there is some evidence that pneumonia, e.g. of goats and calves, mainly occurs in winter. As to strangles itself, it has been possible to obtain the following information. Major H. Allen, R.A.V.C., in a report dated the 14th June 1923 States 'strangles in 1922-23 season was raging in Mona during November, December

January. In Sargodha there were no cases up to 14th February. Strangles started in Sargodha in the middle of February after a period of severe cold wet weather; this is always the case, as it reduces natural resistance of the young stock..... Strangles is still going on in Sargodha and has been kept up, by successive batches of stock received up to the middle of May. Major F. J. Andrews, R.A.V.C., in a letter dated 8 February 1935 states 'As is usual during the winter months here (Sargodha), the heavy rain was followed by a number of cases of Strangles in *nearly all paddocks of susceptible stock*'.* Major W. E. Barry, R.A.V.C. in a report dated the 14 June 1938 states 'There were few deaths up to the end of April (at Sargodha); up to this time the disease was not very severe, probably due to the favourable weather, the winter being mild with little rain'. During a visit to the Punjab in May 1943, I obtained the following information. Major M. F. Keightley, Remount Department (commanding at Mona) said that 'cases are most severe after the Christmas rains of December and January. The disease tends to begin after a heavy downpour, followed by three or four cloudy days with cold wind'. At Sargodha, the veterinary officer and two risaldars gave independent evidence of similar nature and added that the warm dry weather coming at the end of March and in April tends to be followed by a decline in the number of cases. At both depots, the storms are local, sharp, and frequent from January on. Rain is also very localized at Coleyana and Renala, but the amount is rather less in winter than that at the depots.

The problem of why diseases have a seasonal trend is undoubtedly complex. In the case of respiratory disease, two factors at least may be operative on the host, (a) extremes of air temperature and humidity or sudden changes in these elements, and (b) a transient and perhaps seasonal defect in the mechanism of immunity.

It is common knowledge that a more or less sudden cooling of the body through exposure to low air temperature is liable to disturb the function of the respiratory tract, especially when the effects of cold are accentuated by wetting of the body surface through rain and by the action of wind. In man, a similar result may be brought about during the hot dry season by chills induced by excessive radiation or evaporation; cold in the head being by no means uncommon through this cause, when the shade temperature level is over 100°F. Exposure to cold will not only initiate clinical attacks in the more susceptible animals but will also raise the general carrier rate. In consequence, the amount and diffusion of in-

fective material are increased, so that fresh impetus is given to the outbreak and it may continue for some time, possibly in milder form, in spite of an improvement in the weather.

Again, in connection with this first factor, Winslow, Herrington and Nelbach [1942] have shown that substantial drying of the nose and throat occurs at air temperatures of say 70° or 80°F, if the relative humidities at these temperatures are less than 54 and 39 respectively. Boyd and Johnston [1940] have pointed out that a fine adjustment in the water content of the respiratory tract (trachea and lungs) is required to maintain this in health. They showed, in fact, with rats that the water content of the lungs tends to be remarkably constant. During the autumn, however, when the animals were exposed to the first cold spell, there was a significant increase in water content, and this was followed by an equally significant drying of the tract when the hot-water system in their living rooms was turned on. Further, Cralley [1942] has shown in experiments on rabbits that, when the animals are made to inhale very large numbers of bacteria through the medium of droplet nuclei, only a very small percentage reaches the lungs. Under 'normal' conditions (air temperature 26°C. and relative humidity 45 per cent), 80 per cent of the organisms that do reach the lungs are removed within the first hour and over 90 per cent within three hours. When the animals are first subjected to extremes of temperature and humidity (38°C. and 30 per cent humidity, 4°C. and 80 per cent humidity) or first to one extreme and then to another, the rate of removal is temporarily but definitely depressed.

As to the second factor, there is reason to suppose that the animal body undergoes an important modification in susceptibility, which may be seasonal. Wilson [1930] has shown that in mice there occur transient fluctuations in their resistance to *Bact. aertrycke*. These variations in resistance, however, were not definitely seasonal and the author applies the term 'fluctuating immunity' to those 'variations in resistance which occur from time to time and which are due to alterations in the physiological behaviour of the animal dependent on changing environmental conditions'. The nature of the change in question has excited the attention of investigators and is but vaguely understood. It may have an external origin, e.g., it may be dietetic and concerned with the lack of fresh green fodder at certain seasons. Webster & Burn (*loc. cit.*), for instance, noted a higher proportion of *Pasteurella* carriers among rabbits on an 'incomplete' diet than in ones on a 'complete' diet. As is the case with 'chills', there would result a larger amount of infective material available to the population

* The italics are introduced by me, because this statement points to some uniformly acting cause

On the other hand, the change may be intrinsic in origin and connected with seasonal modifications in endocrinal activity.

Apart from the two factors named above, a dry hot atmosphere will promote dust formation, especially when there is some movement of the air, and this by irritating the mucous membranes may assist the penetration of infective material. Rain-fall and moist atmospheric conditions will have an opposite effect. Incidentally, it may be mentioned that, contrary to what is sometimes imagined, haemolytic streptococci are not delicate organisms. Thus, it has been shown that they can survive in dust for weeks and are often abundant in the air of rooms that have been occupied by human beings suffering from streptococcus disease [White, 1936; Brown and Allison, 1937].

By way of comment on these predisposing causes, it may be said that some drying effect on the respiratory tract of young horses may occur at certain seasons, e.g., in May and June at Montgomery, and this adverse effect may be enhanced by an excess of dust in the atmosphere. This, however, is little more than a guess, and the data at hand scarcely permit a serious consideration of the possibility. Again, as to whether there is any transient variation in the animals' resistance, little can be said. If there is, it can hardly be due, in this case, to lack of green food. Of the causes mentioned, the influence of chilling must be regarded as of preponderating importance, especially in young animals, and the evidence produced in this paper supports the statements of observers on the spot. The lack of adequate shelter under winter conditions in the Punjab must put a severe strain on the animal's heat regulation, and it is strongly felt that insufficient attention has been paid to means for remedying this. (The photographs accompanying my previous paper are believed to provide evidence of this.)

The Climatological Atlas of India shows that the absolute minimum temperature in December and January (the coldest months) is around 28°F. at Khushab (Shalpur District), slightly higher at Montgomery but still very low viz., 30°F., while at Ahmednagar it is 40° to 45°F. Mean minimum temperatures for these months are about 40° to 41°F. at Khushab, 42-5°F. at Montgomery and 53°F. at Ahmednagar (see also Table D).

It has been pointed out above that the winter storms of the Punjab and the northerly breeze at this season in the north-west of the province will reinforce the action of cold, but that the more southerly breezes at the time of the summer rains are unlikely to have much damaging effect. It seems likely also that the reason why donkeys usually escape the disease is simply because they have much heavier coats, and not because they are inherently more resistant. Objections to shelters have been raised on the score of cost but all that is really needed are cheap constructions. In the day

time, simple mud-brick walls would suffice to keep off driving wind and rain or a mud-wall enclosure might be provided in each paddock as refuge both by day and night in bad weather. The planting of trees as wind-breaks may also be recommended.

It may thus be said that there seem to be two important factors involved in the epizootiology of strangles, (a) the cold and rain of the Punjab winter, which, perhaps by increasing a normally elevated carrier rate at this season, is largely instrumental in initiating outbreaks and thereby augmenting the dosage of infective matter in the locality; (b) at the depots the introduction of fresh susceptible young stock at a particularly dangerous time, viz. late in the purchasing season. As Allen [1923] says, 'It is impossible to control strangles if.....young stock are received as late as April or May when strangles is usually at its height'. This second factor, however, may be misleading and cause one to overlook the point that the initial stimulus has been unfavourable weather conditions. The relative importance of these two factors will no doubt vary to some extent in different years, and it is unnecessary to hold rigid views. But it is difficult to escape the conclusion that, from the meteorological point of view, the remount depots and horse-breeding farms of the Punjab are unhappily situated.

SUMMARY

Equine strangles, as studied in India, is chiefly a disease of the colder months. In this respect it resembles certain respiratory diseases of man, e.g. scarlet fever, colds, and pneumonia. Thus, it has been shown that in the Punjab the attack rate is significantly higher in the cold season than in the hot and is significantly lower in the summer rainy season. At Ahmednagar (Bombay), where the climate is more equable than in the Punjab, the attack rate also rises significantly during the drier and cooler months and falls during the warmer wet months from April to October.

Further support to the general conclusion is derived from an examination of local meteorological data which in the Punjab show some degree of association between low absolute minimum temperatures and the onset of outbreaks.

The association between weather conditions and strangles is studied to best advantage at the farms where the stock are reared; at the depots, conditions are somewhat artificial and the observations are confused by the fact that the numbers of susceptible stock are mounting fairly rapidly throughout the winter, while by May outbreaks tend to terminate abruptly owing to the shortage of susceptibles. In general, the observations are complicated by the facts that, while outbreaks tend to develop in the cold weather, they may continue

for some time after it has warmed up. On the Punjab farms, it is also to be noted that (1) a secondary rise in the attack rate may occur in May-June when for days on end there is a very hot dry wind with liability to dust storms, and (2) following several seasons of low incidence and resulting increase in the local susceptibility, outbreaks sometimes commence during the hot season.

In the endeavour to control strangles in the Punjab, insufficient attention has been paid to the necessity of protecting young horses from the effects of extreme cold and winter rain.

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A STUDY OF THE VILLAVECHIA REACTION FOR SESAME OIL

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THE systematic studies by Villavechia and Fabris on the utility of the Boudaun test for detecting the presence of sesame oil were carried out in 1893. As no data are available on the relation of the colour developed during the reaction to the quantity of the oil present, a detailed study was carried out to study the effects of various factors on the colour development in the Villavechia reaction and further to determine the possibilities of increasing the sensitivity of the test in general use at present.

EXPERIMENTAL

The Villavechia reaction is usually carried out as follows [A.O.A.C., 1940].

A mixture of 0.1 ml. of 2 per cent alcoholic solution of furfural and 10 ml. of HCl are shaken with 10 ml. of oil sample for 15 seconds and the aqueous layer allowed to separate. Instead of furfural solution 0.1 gm. of finely produced sugar dissolved in 10 ml. of HCl can also be used.

Taking this method of carrying out the test as standard, the effect of different factors was

investigated, introducing appropriate modifications wherever necessary.

For the following studies (when not otherwise mentioned) a 5 per cent solution of sesame oil in groundnut oil was used. Both the sesame and groundnut oils were pressed from pure seeds and guaranteed to be genuine. The free acidity was neutralized, and the oils dehydrated and filtered before use. The red colour of the reaction mixture was compared in the Lovibond Tintometer using 1 cm. cell.

(i) Effect of using different strengths of hydrochloric acid

Fuming HCl (sp. gr. 1.19) was diluted to different strengths as indicated in Table I. To match the colour quantitatively, the aqueous coloured layer was pipetted in a centrifuge tube and centrifuged at 2,500 r.p.m. for 5 minutes. This helped to give a clear solution. The colour was matched soon after carrying out the test. The same solutions were then kept in a refrigerator overnight, and colours were compared to see if there was any change in the colour on longer keeping. The results are given in Table I.

TABLE I

Effect of varying the strength of HCl on the Villavechia reaction

	Sp. gr. of HCl used								
	1.19	1.17	1.16	1.15	1.14	1.13	1.12	1.11	1.10
Colour reading 30 min. after starting the reaction	R 13.9	13.1	9.2	6.5	4.4	3.2	1.9	0.9	0.3
	Y 1.0	1.0	1.0	1.0	1.0	1.0	0.3	0.3	0.3
After keeping overnight in	R 13.2	12.6	8.3	5.7	3.7	2.3	1.0	0.3	0.0
	Y 1.3	1.3	1.3	1.0	1.0	1.0	0.3	0.4	0.4

It can be seen from the result that the higher the strength of the acid the stronger was the colour reaction. Further, it was also necessary to match the colours as soon as possible, as there was a considerable diminution of the intensity even when the solutions were kept at low temperatures.

(ii) *Effect of heat on Villavechia reaction*

(a) In this part of the experiment, 10 gm. of oil and 0.1 ml. of furfural solution were mixed and heated for three minutes to different temperatures shown in Table II. Ten ml. of HCl were then added, shaken and the colours matched.

TABLE II

Effect of heating sesame oil and Furfural mixture

Room temp.	40°	50°	60°	80°	100°C.
R 9.6	9.8	10.3	11.3	9.2	8.5
Y 1.0	1.0	1.1	1.1	1.2	1.4

It was seen that heating to 50°=60° C. increased the intensity of the colour developed. Higher temperatures had the reverse effect.

(b) Similar experiments as described in (a) were carried out, this time first mixing the HCl and oil and heating for three minutes, followed by the addition of furfural reagent.

TABLE III

Effect of heating sesame oil and HCl mixtures

Room temp.	40°	50°	60°	80°	100°C.
9.6	10.3	12.0	9.9	6.8	4.3
1.0	1.0	1.1	1.1	1.3	2.0

It can be seen from the above that heating up to 50°C intensified the sensitivity of the test.

(c) Experiments were next carried out, applying similar heat treatments to the whole of the reaction mixture consisting of oil, furfural reagent and HCl. The results are shown in Table IV.

TABLE IV

Effect of heating Villavechia reaction mixture

Room temp.	40°	50°	60°	80°	100° C.
R 9.7	11.8	14.1	14.3	14.2	9.9
Y 1.1	2.1	2.2	2.4	2.3	2.9

It can be seen from the above that heating up to 50°-80°C. considerably enhanced the intensity of colour. At higher temperatures than the above the colour intensity decreased.

In subsequent studies reaction mixtures were heated to 50°C. for three minutes. The blank did not develop any colour when heated to 50°C. under similar conditions.

(iii) *Effect of using different quantities of sesame oil*

These trials were carried out to see if the colour produced in the reaction mixture was proportional to the quantity of sesame oil present. Samples of 0.5, 1.0, 5.0, 7.0 and 10.0 gm. of the oil mixture containing 5 per cent sesame oil and 95 per cent groundnut oil were taken and the total weight of oil made up to 10 gm. by adding 9.5, 9.0, 7.0, 5.0, 3.0 and 0.0 gm. of groundnut oil respectively. The results are given in Table V.

TABLE V

Effect of using different quantities of sesame oil on the Villavecchia reaction

Temperature used	Quantities of 5% sesame oil taken in gm.					
	0.5	1.0	3.0	5.0	7.0	10.0
Room temp.	R 1.9	4.4	9.9	13.2	14.8	18.7
	Y 0.3	0.3	1.3	1.3	1.4	1.4
50° C.	R 2.7	6.1	11.9	16.7	20.0	26.4
	Y 1.1	2.0	2.2	2.2	2.2	2.3

The results showed that the colour intensity depended on the amount of oil used, but was not in exact proportion to it. For all subsequent work, the quantity of oil to be used was arbitrarily fixed at 10 gm. as in the general practice.

(iv) *Effect of using different sugars*

In the Baudouin test a 1 per cent solution of cane sugar in HCl is employed. Villavecchia replaced cane sugar by furfural. The following experiments were carried out using 1 per cent solution of different sugars in HCl. Results are shown in Table VI.

It can be seen that the colour intensity when manose, glucose and dextrin were used was

TABLE VI

Effect of different sugars on Villavecchia reaction

Reaction temperature	Sugars used							
	Furfural reagent	Lactose	Invert sugar	Dextrin	Manose	Galactose	Glucose	Fructose
Room temp.	R 15.6	16.4	22.9	15.4	15.8	14.9	15.4	20.6
	Y 2.0	2.0	8.0	1.9	2.0	2.0	2.0	8.6
50° C.	R 27.3	27.0	26.2	26.1	26.7	25.0	26.9	24.2
	Y 3.0	2.8	9.7	3.1	2.8	2.7	2.0	9.8

almost similar to that given by furfural reagent. With galactose, it was slightly lower, and with lactose higher. All the above named sugars did not give any blank. With fructose, invert sugar and cane sugar, the readings were much higher when compared to furfural standard, the intensity increasing in the order mentioned. It was also interesting to note that, in case of the three last named sugars, the raising of the reaction temperature did not produce very marked intensity in colour. Further, fructose, invert sugar and cane sugar gave high blanks as indicated below:

	Fructose	Invert sugar	Sucrose
R.	4.3	3.0	2.2
Y.	19.9	10.2	8.0

As there was a considerable proportion of yellow colour, the quantitative reading suffered. Again, with these three sugars, the reading did not remain constant, but the colour rapidly changed from red to dark brown. For all these reasons, it was felt that the replacement of any sugar for furfural was not of any great benefit under practical condition.

To minimize this defect when different sugars are used, instead of using pure HCl, acid containing 0.5 per cent and 1.0 per cent stannous chloride (Soltsein Reagent) was employed. The results are given in Table VII.

It can be seen from Table VII that by the use of stannous chloride, the colour intensity

TABLE VII

Effect of using sucrose in the presence of stannous chloride

Temperature of reaction mixture	Furfural reagent	Using 0.5% stannous chloride		Using 1.0% stannous chloride	
		Blank	Sesame oil	Blank	Sesame oil
Room temperature	R 15.7	1.8	12.6	1.6	14.9
	Y 2.0	9.8	8.0	10.8	9.8

was much reduced. There was still a large proportion of yellow interfering colour. Thus even under these modifications the furfural reagent was to be preferred.

CONCLUSIONS

(1) The intensity of colour developed in the Villavecchia reaction increased with increase in the strength of HCl. Fuming HCl gave the best results.

(2) The colour developed in the Villavecchia reaction increased with the quantity of sesame oil present in the reaction mixture, but it was not exactly proportional to the quantity of oil used.

(3) The sensitivity of the reaction could be greatly enhanced by heating the test mixture for three minutes at 50°C before centrifuging. Higher temperatures did not produce uniform results.

(4) Substitution of furfural by any of the following sugars, viz. lactose, invert sugar, manose, galactose, glucose, fructose, sucrose and dextrin, did not increase the sensitivity of the reaction.

(5) Improved Villavecchia reaction was carried out as follows:

10 gm. of oil were mixed with 0.1 ml. of 2 per cent alcoholic solutions of furfural and 10 ml. of HCl. After shaking, the tubes were kept in a water-bath at 50°C. for three minutes, shaken and the aqueous layer allowed to separate. For the quantitative estimation of colour, the aqueous layer was transferred to centrifuge tubes and then centrifuged at 2,500 r.p.m. for five minutes.

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A COMPARATIVE STUDY OF THE QUALITY OF GHEE FROM COW AND BUFFALO UNDER UNIFORM DIETARY CONDITIONS, INCLUDING HEAVY FEEDING OF COTTON SEED

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THE results of a series of experiments hitherto carried out to study the effects of various food fats on the quantity and quality of milk fat suggest that the quantitative secretion of milk fat can be altered by a dietary fat, but not over any extended period, whereas the physical and chemical constants of milk fat can be permanently modified by the quality of the fat ingested. These observations have, however, been made on the milk fat from cows only. There is an apparent lack of information as to the extent the milk fat of buffaloes is susceptible to both quantitative and qualitative changes due to the character of the foodfat consumed. Only a few recorded data are available which would indicate the comparative influence of the dietary fat on the milk fats of cows and buffaloes when both these species are fed the same production ration [Doctor *et al.*, 1940] or when their production ration is similarly altered by a dietary modification.

In the present paper are reported the results of an investigation carried out to study:

(a) The comparative difference in the physical and chemical constants of ghee, produced from the

milk fats of both cows and buffaloes, when they were receiving the same production ration, and

(b) The relative changes in the physical and chemical constants of ghee, when the concentrate mixture in the production ration was entirely replaced by cotton seed.

Cotton seed is one of the most commonly used concentrates for milch cattle in Bombay Province and the Western Indian States. There is a popular belief in these parts that cows and buffaloes, when fed on cotton seed, give milk richer in fat, and the ghee made from the butter fat of such animals is of superior quality.

EXPERIMENTAL

For the purpose of the investigation 12 healthy animals, six Kankrej cows and six Surti buffaloes, were selected. The selected animals were approximately similar in age, number and stage of lactation. They were divided into two groups, 'experimental' and 'control'. In the experimental group were taken four cows and four buffaloes, and in the control group, two cows and two buffaloes. The rations for the two groups of animals are set out in Table I.

TABLE I

Statement of rations for the experimental and the control groups of animals

Particulars	Experimental group	Control group
For maintenance	(1) Chopped jowar hay— <i>ad lib</i>	(1) Green Guinea grass 10 lb. (2) Chopped jowar hay— <i>ad lib</i>
For production	(2) 1 lb. of cotton seed per 2 lb. of milk yield	(3) 1 lb. of the following concentrate mixture per 2 lb. of milk yield. <div style="text-align: right; margin-right: 20px;"> Parts Wheat bran 3 Groundnut cake 2 <i>Thu cheni</i> 1 Cotton seed 3 Crushed guar 1 </div>

The animals in both the groups received 2 oz. of common salt per animal per day.

It may be seen from the statement of the two rations that, while cotton seed constituted the sole production ration for the 'experimental' animals, its quota was barely 33 per cent for the production purpose in the case of 'control' animals. As a result of this quantitative difference in the cotton seed content, it has been calculated that the animals in the experimental group were consuming almost double the amount of fat (ether extract) in their production ration when compared with the animals in the control group.

After the animals in the two groups got adjusted to their respective rations, for three consecutive days in a month, the mixed morning and evening milk from each individual animals was curdled and separately kept. Three days collections of curdled milk from each animal were then mixed on the fourth day and churned for butter. The butter was immediately converted into ghee by melting in a suitable (tinned) brass vessel over a slow wood fire. The experiment was started in the month of May 1942 and was concluded at the end of November 1942. During the course of these months, seven approximately monthly lots of ghee were prepared from each animal. Within a month of their preparation, a sample from each was subjected to physical and chemical examination. Altogether 84 samples of ghee were examined. The following constants were determined strictly according to the methods described in the British Standard Methods for Chemical Analysis of Butter [1938] and in the Report of the Analytical Methods Committee of the Society of Public Analyst [1936]:

- (1) Butyro refractometric value at 40°C, (2) Saponification value, (3) Reichert Meissl value (4) Kirschner value, (5) Polenske value and (6) Acid value calculated as oleic acid.

The total number of 84 samples of ghee analysed can be divided into the following categories according to the number of groupings, species and dietary treatments:

28 samples from cows on cotton seed feed	Experimental group
28 samples from buffaloes on cotton seed feed	
14 samples from cows on concentrate mixture	Control group
14 samples from buffaloes on concentrate mixture	

RESULTS AND DISCUSSION

In order to save paper the detailed analytical data are not given. In Table II is presented only the mean value of the total number of the determinations of the different constants in ghee from groups of cows and buffaloes under the experimental and the control feedings. In the same table, the coefficient of variation and the standard error of each mean are also shown.

TABLE II

Constants of ghee from cows and buffaloes under identical dietary treatments

Species	Experimental			Control		
	Mean	Coef. of variation	Standard error	Mean	Coef. of variation	Standard error
<i>B. R. value</i>						
Cow	43.3	0.91	0.074	42.0	1.47	0.167
Buffalo	42.6	1.54	0.123	40.9	0.96	0.104
<i>Sap. value</i>						
Cow	231.0	1.13	0.478	225.2	0.87	0.523
Buffalo	224.1	1.15	0.457	232.6	0.75	0.465
<i>R.M. value</i>						
Cow	23.7	10.40	0.460	25.0	6.00	0.407
Buffalo	23.1	8.37	0.455	34.7	6.80	0.430
<i>K. value</i>						
Cow	20.2	11.10	0.440	21.4	6.23	0.369
Buffalo	25.2	7.92	0.399	30.3	6.88	0.579
<i>P. value</i>						
Cow	1.20	12.58	0.028	1.5	17.00	0.060
Buffalo	0.94	8.62	0.015	1.3	15.62	0.054
<i>Acid value</i>						
Cow	0.47	17.02	0.015	0.56	25.18	0.027
Buffalo	0.31	17.42	0.010	0.41	64.39	0.050

Cow ghee vs. buffalo ghee. The data in Table II offer a clear picture of the impression made by an identical diet on the milk fat of cows and buffaloes. It may be seen that, under both experimental and control feeding, the B.R., P. and Acid values are significantly higher, and such constants as Sap. value, R.M. value and K. value are significantly lower in the ghee from the milk fat of cows than that of buffaloes. These results are in agreement with the findings of Doctor *et al.* [1940] who have determined the physical and chemical constants of ghee from different breeds of cows as well as from Murrah buffaloes, kept under similar conditions of feeding and management. These results thus lead to the conclusion that even under identical dietary and management there is a distinct species difference in the character of the milk fat secreted by the cow and the buffalo. The B.R. and Sap. values suggest that in the milk fat of the buffalo the proportion of the fatty acids of higher molecular weight is less than that in the milk fat of the cow. This is also supported by the higher R.M. value. The higher K. value of buffalo ghee shows that the milk fat of this species is richer in butyric acid, but its proportion of water insoluble volatile acids is comparatively lower than that of the milk fat of the cow as is evident from the P. value determined. The free fatty acid content is higher in the milk fat of the cow than that of the buffalo.

The effect of heavy feeding of cotton seed. The effect of the replacement of the concentrate mixture in the production ration entirely by cotton seed on the constants of ghee from cows and buffaloes is shown in Table III.

TABLE III

The effect of heavy feeding of cotton seed on the physical and chemical constants of ghee

Species	Mean values		Diff. in mean values	Per cent higher (+) or lower (-) value
	Experimental	Control		
Cow	43.3	B. R. value 42.6	0.7	+1.6
Buffalo	42.6	40.9	1.7	+1.6
Cow	221.9	Sap. value 225.2	3.3	-1.5
Buffalo	224.1	232.6	8.5	-3.8
Cow	23.7	R. M. value 25.0	1.3	-5.5
Buffalo	28.1	34.7	6.6	-23.5
Cow	20.2	K. value 21.4	1.2	-5.9
Buffalo	26.2	30.3	5.1	-20.2
Cow	1.30	P. value 1.50	0.30	-25.0
Buffalo	0.94	1.30	0.36	-38.3
Cow	0.47	Acid value 0.56	0.09	-19.2
Buffalo	0.31	0.41	0.10	-32.3

It is apparent from the data in Table III that the qualitative secretion of milk fat in buffaloes is comparatively more susceptible to dietary modification than that in cows.

The limits of the variation in the physical and chemical constants of ghee. The investigation has afforded an opportunity to record the limits of variations in the constants of ghee under the present experimental conditions. In Tables IV and V the minimum and maximum values of different constants of ghee from cows and buffaloes are respectively set out. In these tables, similar figures collected by Doctor, *et al.* [1940] are included for a comparative study.

TABLE IV

Limits of variations in the constants of ghee from cow

Constants	Experimental		Control		Doctor, <i>et al</i>	
	Min.	Max.	Min.	Max.	Min.	Max.
B. R. value	41.9	44.3	41.9	43.1	40.0	45.2
Sap. value	214.6	227.9	221.6	227.9	219.0	230.0
R.M. value	18.7	28.4	23.0	27.3	23.0	30.0
K. value	16.0	24.3	19.2	23.8	17.0	24.2
P. value	1.0	1.4	1.0	1.9	1.9	3.0

TABLE V

Limits of variation in the constants of ghee from buffalo

Constants	Experimental		Control		Doctor, <i>et al</i>	
	Min.	Max.	Min.	Max.	Min.	Max.
B.R. value	41.2	43.6	40.2	41.4	42.5	43.6
Sap. value	218.8	228.6	230.0	235.6	229.0	239.0
R.M. value	23.7	33.4	33.5	37.9	30.2	31.7
K. value	22.3	29.2	27.4	34.0	26.6	26.8
P. value	0.8	1.1	1.0	1.6	1.5	2.0

The wide variability in the constants as evident from the data in Tables IV and V emphasizes the limitation of the chemical and physical methods used at present for testing the purity and for the grading of ghee. It would, however, be of interest for future research to find out the possible correlation between the variation in the constants and such factors as the keeping quality and the biological value of ghee which is being investigated in this laboratory.

SUMMARY

Experiments have been conducted to study the effect of identical diet on the physical and chemical constants of ghee prepared from the milk fat of Kankrej cows and Surati buffaloes. The results

show that the Butyro Refractometric, Polenske and Acid values are significantly higher and the Saponification, Reichert Meissl and Kirschner values are significantly lower of the ghee from the milk fat of the cow than that of the buffalo.

The replacement of the concentrate mixture in the production ration entirely by cotton seed shows that in the ghee from both the cow and the buffalo the B.R. value increases, but all other constants show a decrease. The qualitative change brought about in ghee by cotton seed feeding is more pronounced in buffalo ghee than in cow ghee.

The limits of variations in the constants of ghee from cows and buffaloes under the present condi-

tions of study have been discussed, along with the data of other workers.

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TUBERCULOSIS IN BUFFALOES

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(With Plate XI)

TUBERCULOSIS in buffaloes is mentioned by several authors. Kutchukoff [1909] noted the frequent occurrence of the disease in draught buffaloes in the Balkans, Turkey and some parts of Asia. Tuberculosis is stated to be more common in the male than in the female. He adds that the disease is rare in milch buffaloes even when housed for years in the same byre as tuberculous cows. He tuberculin-tested 20 milch buffaloes and 24 milch cows stalled together and found that 40 per cent of the cows and only one milch buffalo reacted. He observed that tuberculin gave a strong reaction in positive cases in the buffalo. Mitter [1910] reports a case of tuberculosis in a buffalo in Calcutta, with lesions present only in the lungs. Eber [1917] refers to buffaloes in the abattoir at Budapest as being affected with tuberculosis to the extent of only 0.18 per cent. Piot Bey [1917] observed that in the abattoir at Alexandria, tuberculosis was not seen in young buffalo calves, but only in animals three to five years old. About 60 per cent of old animals, particularly females, were affected, and 1 to 2 per cent of these showed large and multiple tuberculous lesions. In the abattoir at Cairo, tuberculosis is frequently seen in young buffaloes and 7 per cent of all buffaloes slaughtered are found affected, mainly with lesions in the thorax and uncommonly in the abdominal organs. Tuberculous lesions in the buffalo are said to resemble those usually met with in cattle, and some portions of the glands have

been found calcified. Soparkar [1925] tested the susceptibility of different breeds of Indian cattle and buffaloes to bovine tuberculosis and found that buffaloes were apparently more susceptible than cattle. By subcutaneous inoculation of 50 mg. virulent bovine culture in five to six-month-old calves and 14-month-old buffalo calves he obtained a greater death rate in buffalo-calves. The same writer [1926] observed a case of tuberculosis in a male-buffalo at Mukteswar in India. This animal reacted strongly to the double-intradermal tuberculin test and on post-mortem advanced caseating tuberculosis lesions were found in the mediastinal and bronchial glands. Soparkar and Dhillon [1931] examined 1,116 animals (cows, bullocks and buffaloes) in the slaughter house at Lahore and found 255 animals, i.e. 22.85 per cent, infected with tuberculosis. The incidence of tuberculosis in cows, bullocks and buffaloes was 21.3 per cent, 31.6 per cent and 23.6 per cent respectively. Naik [1932] described a case of tuberculosis in a female Jafferbadi buffalo. The animal was in poor condition, and had loose bowels, a short husky cough, impaired appetite, pale mucous membranes and accelerated respiration. On post-mortem there was extreme emaciation, and tuberculous lesions were present in both lungs, and the mediastinal, bronchial, submaxillary, posterior mammary and mesenteric glands, as well as in the liver. Carrano [1934] considers that in Egypt tuberculosis is more prevalent

among buffaloes than is generally believed, especially in lower Egypt where buffaloes are housed under unsatisfactory condition. His data on incidence are limited to information obtained from public abattoirs. Statistics collected at the abattoir at Alexandria on animals from lower Egypt showed that about 60 per cent of aged buffaloes and 9 per cent of the calves slaughtered were affected with tuberculosis, especially the pulmonary form. In the abattoir at Cairo the incidence was considerably less and this is attributed to the fact that most of the animals slaughtered came from upper Egypt or the Sudan where housing conditions are reasonably good. Carpano tested 350 apparently healthy buffaloes (348 female and 2 male) by the intradermal and subcutaneous methods; 51 reacted to the intradermal test: of these 11 gave positive reactions when subsequently tested by the subcutaneous method and had tuberculous lesions on post-mortem examination. Carpano noted that tuberculin, both in buffaloes and cattle, produced a similar type of reaction, except that the subcutaneous injection of tuberculin in buffaloes was often followed by a local reaction and the maximum temperature was not reached until 30 hours after injection; sometimes the local reaction is so severe as to cause lameness and inappetence, and transitory decrease in milk yield. It is concluded that buffaloes are more sensitive to tuberculin than cattle and the subcutaneous test is recommended in the field owing to the possibility of non-specific reactions after intradermal injection. Bubermann [1934], in Java, reports that a skin disease of buffaloes characterized by the presence of 'tubercles' was examined by cultural and animal inoculation tests for tuberculosis but with negative results. He also refers to an atypical strain of the tubercle bacillus isolated from a buffalo. Topacio [1935] states that clinical tuberculosis has not been recorded in buffaloes in the Philippines, but that they readily die of tuberculosis after experimental inoculation. Buianos [1936], in Greece, states that buffaloes are considered less susceptible to tuberculosis than cattle. Lobel, Van der Schaaf and Roza [1936] isolated three strains of tubercle bacilli from the water-buffalo and found them to be of bovine type. These strains had a low virulence for small laboratory animals and gave a week initial growth on media. The report of a Committee on Diseases of the Philippine Veterinary Medical Association [1937] states that three cases of buffalo tuberculosis were diagnosed by microscopical and bacteriological methods. One of these was shown by

animal and cultural tests to be due to the bovine type of bacillus. Topacio and Coronel [1941] demonstrated that the native Carabao (domestic buffalo) of the Philippines is experimentally susceptible to tuberculosis and that a healthy carabao placed in contact with an artificially infected carabao contracted the disease. They add that the carabao is susceptible to natural infection under ranch conditions and that fatal termination occurred when the animal's resistance is lowered by an inter-current disease such as surra. Hadwen [1942] reports an outbreak of tuberculosis in a large herd of buffaloes in the National Buffalo Park at Wainwright (U.S.A.). In 1907 the herd consisted of 142 buffaloes. From 1911 to 1923, there was no outward indication of tuberculosis in the herd, but in 1923, it was decided to destroy some of the animals. In the first three kills (1923-29) the oldest animals in the herd were slaughtered, and the percentage of tuberculous affection was the highest in these 3 kills, viz., about 72 to 77 per cent animals. In the final slaughter in 1930, there was a marked drop in the percentage with tuberculous lesions, viz. 30.14 per cent: in this kill animals of all ages including calves were slaughtered. Among animals slaughtered in 1923, several cases of generalized tuberculosis were noted. This worker observes that the incidence of tuberculosis did not prevent the herd increasing in size.

The writer encountered a case of tuberculosis in a she-buffalo in Lahore. The animal, although in excellent bodily condition, showed a high temperature, dyspnoea, an occasional cough, a jugular pulse and oedema of the dewlap. The owner stated that the buffalo was a heavy milker and that she suddenly went dry about two days before admission to the hospital. The case was diagnosed as traumatic pericarditis with pneumonia as a complication; death occurred on the 3rd day. Post-mortem examination showed extensive bilateral pulmonary lesions with tuberculous pneumonia of the anterior lobes. The lesions varied in size from a pea to a tennis ball. The contents of the larger tubercles were dissipated, with cavitation, (Plate X figs. 1 and 2). The mediastinal and bronchial glands were enlarged and extensively caseated. No other part of the body was found affected. This case indicates that the bodily condition may at times be so well maintained that disease may not be suspected, though it may be advanced.

Data concerning the types of tubercle bacilli isolated from the buffalo are extremely meagre, and this investigation on abattoir material was,

therefore, undertaken to provide such data. Since the data obtained from one province alone might not be applicable to the whole of India, the investigation was centred at three widely separated places, viz., Izatnagar (U. P.), Bombay City and Calcutta and included the examination of buffaloes from both rural and urban areas. In all, 500 buffaloes were examined, viz., 250 at Izatnagar in 1940, 120 at Kuria in Bombay City in 1941, and 130 buffaloes at Calcutta in 1942. The animals presented for slaughter at these places had no doubt been brought up under different living conditions and were thus to some extent representative of both town and village animals.

TECHNIQUE

Material from an abattoir near Izatnagar was brought to the laboratory and examined by culture and animal inoculation; at Bombay and Calcutta material was straightaway inoculated into guineapigs which were subsequently examined at the Institute. Since primary cultures from tuberculous lesions were not obtained on Dorset egg medium in the first attempts at Izatnagar, the original material was in all further cases inoculated into guineapigs from which cultures on Dorset egg were successfully raised.

INCIDENCE OF INFECTION

At Izatnagar abattoir, about 30 to 40 buffaloes are slaughtered daily, the animals being brought from villages within a 50 mile radius. Buffaloes of both sexes, usually 6-10 years, are slaughtered and are mostly in good condition. The post-mortem examination was necessarily confined to the lungs and to the thoracic, pharyngeal, sub-maxillary and mesenteric glands.

In Bombay, about 20 female buffaloes are slaughtered daily. These animals are in good condition and are disposed of by city dairies when dry or nearly so. The unfavourable conditions of maintenance render them more liable to tuberculosis. In Calcutta the buffaloes slaughtered are in fairly good condition and they are probably of mixed rural and urban origin.

The incidence of the disease among the animals examined was as follows:

	Number	Tuberculous	Percentage
Izatnagar . . .	250	6	2.4
Kuria . . .	120	16	13.3
Calcutta . . .	130	3	2.3

In stall-fed buffaloes of Bombay City, the incidence was high compared with that of pasture-

fed animals bred in the rural districts around Izatnagar. This percentage does not, perhaps, represent the average incidence of tuberculosis in buffaloes in India, as many of these animals may have been sent for slaughter because they were obviously diseased, and disposal by slaughter proved a convenient means of removal.

Table I shows the age, sex, and details of infection of the 25 tuberculous buffaloes:

TABLE I

Number	Place	Sex	Age (Years)	Site of lesions
1	Izatnagar	F.	8	Bron. Calcified
2	...	M.	8	Post. Med.
3	...	F.	6	Bron. Calcified
4	...	F.	10	Bron. Calcified tubercles
5	...	M.	8	Post. Med. tubercles
6	...	F.	6	Ante-Med. Calcified tubercles
7	Bombay	F.	9	Med. & Bron
8	...	F.	7	Bron., cascading tubercles
9	...	F.	5	Post. Med. & Rt. lung, numerous tubercles
10	...	F.	8	Bron. & Post. Med., calcified tubercles
11	...	F.	6	Rt. Bron. & Rt. lung, numerous cascading tubercles
12	...	F.	8	Post. Med. & Rt. Bron., calcified tubercles
13	...	F.	7	All thoracic glands & both lungs
14	...	F.	8	Lt. Bron. calcified
15	...	F.	10	Lt. Bron. calcified
16	...	F.	10	Post. Med.
17	...	F.	8	All thoracic glands & Lt. main lobe lung
18	...	F.	3	Lt. Bron. Cascading tubercles
19	...	F.	9	Post. Med. gland, calcified tubercles
20	...	F.	7	Lt. Bron., calcified tubercles
21	...	F.	6	Lt. Bron., calcified tubercles
22	...	F.	10	All thoracic glands, lungs free. Calcified tubercles
23	Calcutta	F.	7	All thoracic glands and left lung, cascading tubercles
24	...	F.	8	Both Bron., Calcified tubercles
25	...	F.	10	Lt. Bron., Calcified tubercles

Bron. = bronchial glands

Med. = mediastinal

As may be seen, lesions were confined to the thoracic cavity either in the glands alone or in the lung tissue, suggesting that the method of infection is by the respiratory tract. The only record in this country of infection arising by any other route is that of Naik where infection was

presumably by the alimentary route. Most of the diseased animals were in fairly good condition, and in some the condition was excellent. Calcification of the lesions (15 out of 25 cases) was not uncommon. Of the six cases at Izatnagar, three alone yielded tubercle bacilli in culture. Whilst 16 cases were detected at Bombay and confirmed microscopically, material from only eight cases was inoculated into guineapigs. Of the three cases in Calcutta the organism was recovered from two: thus 13 strains were available for typing.

TYPING OF TUBERCLE BACILLI FROM BUFFALOES

Cultural. Tubes of plain egg (Dorset medium) inoculated from the preclear and sub-lumbar glands and spleen of affected guinea pigs were incubated at 37°C. Growth appeared after five weeks or in some cases after nine

weeks. The average number of days for appearance of growth in primary culture was 53 days. The second and third generation cultures grew more quickly but growth was not prolific. Culturally, the buffalo tubercle strains appeared to be typically dysgonic and were nearly always restrained by added glycerin, with one exception (strain 7), which grew equally in plain and in glycerinated egg media.

Animal inoculation. Guinea-pig inoculation with organ-suspension from the primary lesion appeared to indicate that the virulence of the organism was lower than that usual for bovine types, since in some cases five months elapsed before death. Experiments with the second and third generation cultures indicated, however, that the virulence was of standard type. Table II shows the results of virulence tests in rabbits, guinea-pigs and fowls:

TABLE II

Strain	Animal	Inoculum	Result (days)	Post mortem
1	G. pig	Gland suspension 0.5 c.c. S/c	K. 105	G. T.
	"	" " " "	K. 66	G. T.
	"	1.0 mg. of 2nd gen. cult. S/c	K. 60	G. T.
	"	0.1 mg. " " "	D. 67	G. T.
	Rabbit	0.1 mg. " " I/v	D. 22	G. T.
	"	" " " "	D. 38	G. T.
	"	5.0 mg. " " S/c	D. 101	G. T.
	Fowl	1.0 mg. " " I/v	D. 54	Spirochaetosis. No T. B.
	"	0.01 mg. " " "	D. 190	Death due to other causes. No T. B.
2	G. pig	0.5 c.c. of gland suspension S/c	K. 64	Enlarged and caseous local and lumbar gld.
	"	" " " "	K. 99	" " " "
	"	1.0 mg. 2nd gen. cult. S/c	D. 54	" " " "
	"	0.1 " " " "	D. 91	G. T.
	Rabbit	0.1 mg. " " I/v	D. 52	G. T.
	"	0.01 mg. " " "	D. 38	G. T.
	"	5.0 mg. " " "	D. 114	G. T.
	Fowl	1.0 mg. " " "	K. 248	No T. B.
	"	0.01 mg. " " "	D. 10	No T. B.
3	G. pig	0.5 c.c. gld. suspension S/c	K. 94	G. T.
	"	" " " "	K. 59	G. T.
	"	1.0 mg. 2nd gen. cult. S/c	D. 24	Peritonitis
	"	0.1 mg. " " "	D. 66	G. T.
	Rabbit	0.1 mg. " " I/v	D. 25	G. T.
	"	0.01 mg. " " "	D. 29	G. T.
	"	5.0 mg. " " S/c	D. 50	G. T.
	Fowl	1.0 mg. " " I/v	D. 32	Ranikhet disease
	"	0.01 mg. " " "	K. 248	No T. B.
4	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 58	G. T.
	"	0.1 mg. " " I/v	D. 15	T. B. ?
	"	0.01 mg. " " "	D. 19	T. B. ?
	"	0.1 mg. 4th gen. " " "	D. 30	G. T.
	"	0.01 mg. " " "	D. 33	G. T.
	G. pig	1.0 mg. 2nd gen. " S/c	D. 42	G. T.
	"	0.1 mg. " " "	D. 50	G. T.

S/c=Subcutaneously.

I/v=Intravenously.

D.=Died

K.=Killed

G. T.=Generalized tuberculosis

*Tuberculosis in Buffaloes*TABLE II--*contd.*

Strain	Animal	Inoculum	Result (days)	Post-mortem
5	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 31	T. B. ?
	"	0.1 mg. " " I/v	D. 16	T. B. ?
	"	0.01 mg. " " "	D. 19	T. B.
	"	0.1 mg. 4th gen. " I/v	D. 27	G. T.
	"	0.01 mg. " " "	D. 35	G. T.
	G. pig.	1.0 mg. 2nd gen. " S/c	D. 39	G. T.
	"	0.1 mg. " " "	D. 52	G. T.
6	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 82	G. T.
	"	0.1 mg. " " I/v	D. 27	G. T.
	"	0.01 mg. " " "	D. 22	G. T.
	G. pig	1.0 mg. " " S/c	D. 35	G. T.
	"	0.1 mg. " " "	D. 51	G. T.
7	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 20	T. B. ?
	"	0.1 mg. " " I/v	D. 21	T. B. ?
	"	0.01 mg. " " "	D. 33	G. T.
	G. pig	1.0 mg. " " S/c	D. 37	G. T.
	"	0.1 mg. " " "	D. 46	G. T.
8	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 125	G. T.
	"	0.1 mg. " " I/v	D. 28	G. T.
	"	0.01 mg. " " "	D. 10	No T. B.
	"	0.1 mg. 4th gen. " "	D. 8	No T. B.
	"	0.01 mg. " " "	D. 42	G. T.
	G. Pig	1.0 mg. 2nd gen. " S/c	D. 71	G. T.
	"	0.1 mg. " " "	D. 32	G. T.
9	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 109	G. T.
	"	0.1 mg. " " I/v	D. 25	G. T.
	"	0.01 mg. " " "	D. 30	G. T.
	G. pig	1.0 mg. " " S/c	D. 51	G. T.
	"	0.1 mg. " " "	D. 52	G. T.
10	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 81	G. T.
	"	0.1 mg. " " I/v	D. 26	T. B. ?
	"	0.01 mg. " " "	D. 7	No T. B.
	"	0.1 mg. 4th gen. " "	D. 33	G. T.
	"	0.01 mg. " " "	D. 33	G. T.
	G. pig	1.0 mg. 2nd gen. " S/c	D. 67	G. T.
	"	0.1 mg. " " "	D. 61	G. T.
11	Rabbit	5.0 mg. 2nd gen. cult. S/c	D. 57	G. T.
	"	0.1 mg. " " I/v	D. 41	G. T.
	"	0.01 mg. " " "	D. 44	G. T.
	G. pig	1.0 mg. " " S/c	D. 49	G. T.
	"	0.1 mg. " " "	D. 61	G. T.
12	Rabbit	0.1 mg. 2nd gen. cult. I/v	D. 35	G. T.
	"	0.01 mg. " " "	D. 54	G. T.
	"	5.0 mg. " " "	D. 73	G. T.
	G. pig	1.0 mg. " " "	D. 57	G. T.
	"	0.1 mg. " " "	D. 60	G. T.
	Fowl	1.0 mg. " " I/v	K. 180	No T. B.
	"	0.01mg. " " "	K. 180	No T. B.
13	Rabbit	0.1 mg. 2nd gen. cult I/v	D. 42	G. T.
	"	0.01 mg. " " "	D. 56	G. T.
	"	5.0 mg. " " "	D. 74	G. T.
	G. pig	1.0 mg. " " "	D. 53	G. T.
	"	0.1 mg. " " "	D. 58	G. T.
	Fowl	1.0 mg. " " I/v	K. 160	No T. B.
	"	0.01 mg. " " "	K. 160	No T. B.

S/c=Subcutaneously

I/v=Intravenously

D=Died

K=Killed

G. T.=Generalized tuberculosis.



FIG. 1. Lung. Caseating tubercles of various sizes



FIG. 2. Lung. An extensive caseating tubercle with numerous smaller tubercles. The lesions are breaking down to show cavity formation.



Showing swelling of hock, knee or fetlock joints

It will be seen from the Table II that:

After 1.0 mg. S/c guinea pigs survived for 99/71 days, mean 51 days.

After 0.1 mg. S/c guinea pigs survived for 32-91 days, mean 57 days.

After 0.1 mg. I/v rabbits survived for 21-52 days, mean 31 days.

After 0.01 mg. rabbits survived for 22-56 days, mean 37 days.

After 5.0 mg. S/c rabbits survived for 50-125 32-81 days, mean 57 days.

Fowls inoculated intravenously with 0.1 and 0.01 mg. of culture showed no evidence of tuberculosis when examined six months later.

Thus, the results of pathogenicity, and cultural tests indicate that all these 13 strains are of bovine type and that second and third generation cultures of standard virulence.

SUMMARY

The available literature on tuberculosis in buffaloes is reviewed.

Examination of 500 buffaloes slaughtered in three abattoirs in the United Provinces, Bombay and Bengal yielded 25 cases of tuberculosis. *Mycobacterium tuberculosis* was isolated from 13 of these. Cultural and biological studies showed them to be of the bovine type and the virulence was standard only after the strains had been cultured.

ACKNOWLEDGEMENTS

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SOME OBSERVATIONS ON 'WAH' OF GOATS AND SHEEP IN SIND

By H. S. BAWA, M.R.C.V.S., Veterinary Investigation Officer, Sind, Karachi

(Received for publication on 21 September 1943)

(With Plate XI)

THE disease known as *Wah* occurs in certain districts of Sind, viz. Nawabshah, Tharparkar, Sukkur, Hyderabad and Dadu.

The local name *Wah* means in Sindhi rheumatism, i.e. a painful swelling of the joints. The disease occurs throughout the year, but its incidence seems to be more common during the month of February. Some herdsmen believe that the new shoots of grass arriving with the onset of the summer and winter rains are the aetiological factor, especially when they are grazed on with the morning dew on them. Mortality varies in different outbreaks from 10 to 30 per cent.

The condition was first noticed in Nawabshah in 1937 both in adults as well as kids, and a three-day old affected kid was purchased for pathological

study, which constituted the start of it investigation.

The clinical picture, course and morbid anatomy will be described briefly.

SYMPTOMS

They are both systemic and local and are well marked so that the shepherd is quick in recognizing them. It is usually a chronic disease, but when acute, there is a sharp rise of temperature and a total or partial arrest of milk secretion, followed by great debility and death before any local lesions develop.

The acute form of the disease is rarely seen, while the sub-acute form with local lesions is what generally met with. In sub-acute cases

the primary local lesion is generally in the udder, which becomes hot, swollen and painful, while the milk becomes either thick or thin and watery, occasionally blood-tinged with fibrin clots and is greatly reduced in quantity. A painful swelling of the knee and hock joints appears three or four days after the udder complications, giving rise to acute lameness. The swelling of the udder subsides within seven to ten days and is followed by an atrophy of the gland. The milk secretion does not return to normal. The few drops which can be squeezed out with great difficulty are greatly altered in consistency and appearance, having become either thick or watery and flocculent. In about three months the swelling of the joints decreases and the lameness disappears; the mammary gland becomes soft and on palpation may be found to contain indurated areas. Usually only one quarter of the udder is involved. When the whole udder is involved, there is no secretion of milk in the affected quarters on the next kidding, with the result that the young ones die of starvation. Lambs and kids may be infected in *utero* and show swelling of the hock, knee or fetlock joints at birth and are unable to stand (Plate XI). The majority of them die after a few days.

Sometimes the disease affects the mammary glands only. In one outbreak 5 per cent of the affected goats and sheep had inflammatory eye lesions, viz., conjunctivitis, corneal opacity and the presence of highly-injected minute blood vessels in the sclera, immediately around the cornea. In this particular outbreak 20 per cent of the animals showed obvious lachrymation. The lactating animals are the most susceptible, but males and the non-lactating animals may also be affected. Young animals are less susceptible than adults.

It has been observed that abortion is quite common among goats suffering from *Wah*, the percentage of abortion varying from 5 to 20 per cent.

It may be mentioned here that generally the disease is seen in goats only, while sheep in the same area, though grazing and living with affected goats, seem to remain free. Only one outbreak has been recorded in which both goats and sheep were seen to be affected. In the experimental animals the symptoms observed were the same as those noticed in natural cases. Under natural and experimental conditions only a certain percentage of animals contract the disease.

MORBID ANATOMY

Autopsy on ten natural cases of the disease and three experimental goats revealed that the main changes were confined to the knee and mammary

glands. The postmortem findings are summarized below in detail:

Knee joints. The swollen joints were painful and soft on pressure, and contained an excess of fluid. The bursae were thickened and there was fibrositis.

Mammary gland. The gland was atrophied and soft and had indurated areas. In acute cases the gland was inflamed and showed areas of necrosis. The mammary lymphatic glands were also enlarged in acute cases.

In some cases the liver appeared to be enlarged; sometimes there was infestation with liver-flukes, while at other times no parasite could be found. Other organs were normal, except that the stomach and intestines showed a varying degree of helminthic infestation, among which amphistomes, *Haemonchus contortus*, and *Trichuris ovis* were predominant.

HISTOPATHOLOGY

Knee joints. There was peri-arthritis, with proliferation of fibrous tissue. In some cases only bursitis and superficial fibrositis were seen.

Udder. Inflammatory changes with multiple abscesses were noticed in experimental animals, which had been given infective material through the teat canal. These changes were also noticed in some natural cases. In some cases, advanced hyperplasia of the fibrous tissue with monocytic infiltration round the acini was seen, and in others slight monocytic infiltration round the acini and very slight fibrosis were noticed.

Liver. Biliary and portal cirrhosis, extensive haemorrhagic areas (haemorrhagic infarcts) and verminous nodules, eosinophile infiltration, associated with necrotic changes were observed. No helminth parasites could be detected in sections.

BACTERIOLOGY

Examinations of smears of blood, milk and films from the cut surface of the mammary gland and from knee joint fluid repeatedly proved negative for pathogenic micro-organisms and protozoa. In most cases the cultural examination of different organs, viz., liver, spleen, kidney, mammary gland, knee joint, milk and urine failed to reveal any pathogenic organisms.

In one case coliform organisms were isolated from the liver, spleen, kidneys, heart-blood and knee-joints, but they were of no pathogenic significance, as both the young and old were seen to be affected alike and there was nothing to suggest navel-ill or joint-ill. In one instance staphylococci were isolated from the joint fluid. These presumably were contaminants, as no sign of pus or abscess formation has ever been seen in the knee joints.

Milk, blood and urine from affected animals were culturally examined for *Brucella abortus*.

and *Brucella melitensis* with negative results. Numerous samples of blood sera were examined and found to be negative on agglutination test for *Brucella* infection.

TRANSMISSION EXPERIMENTS

These experiments were conducted with a view to finding out the infectious nature of the

disease. For these experiments, naturally affected goats were brought to Karachi in order to have a fresh supply of material at hand.

Experiment No. 1

Four healthy goats were used, and the material was obtained from the donor goat, No. DI., brought from Jamesabad.

TABLE I
Material obtained from donor goat No. DI

Expt. goat No.	Date	Route	Material	Dose	Remarks
1	27-4-39	Intrav.	Blood	5 c.c.	Shown rise of temp. to 103.8°F. on 7th day and again to 104°F. on 14th day. Kept under observation for two months but showed no signs of disease
2	Do.	Subcut. & orally for 7 days	Milk	10 c.c.	Kept under observation for two months, but showed no signs of the disease
3	Do.	Dronehed for 8 days	Faeces and urine	15 c.c.	Ditto
4	Do.	Subcut.	Knee-joint fluid.	2 c.c.	Ditto

The results of these experiments (Table I) suggest that the disease is not transmissible by the above routes with the material used.

Experiment No. 2

Since in experiment No. 1 intravenous inoculation of blood in goat No. 1 showed some reac-

tion, it was decided to repeat blood inoculation. Three healthy goats were used for this experiment.

No definite conclusions could be drawn from the experiments (Table II), as the animals did not develop the disease while under observation for two months and a half.

TABLE II
Material obtained from donor goat No. D2

Expt. goat No.	Date	Route	Material	Dose	Remarks
5	6-5-41	Intrav.	Blood	10 c.c.	Shown thermal reaction to 104°F. on 23rd day after injection, which returned to normal after two days. Goat aborted on 21st June.
6	Do.	Intrav.	Do.	10 c.c.	Same as above, except that goat did not abort.
7	Do.	Subcut.	Do.	10 c.c.	On 2nd day of inoculation temp. rose to 105.3°F. due to abscess formation. Abscess burst on 4th day, and temp. then returned to normal.

Experiment No. 3

This experiment was designed to explore the possibility of milk being the carrier of an infective agent; also to see if the teat canal would serve as the route of infection, since milk given subcutaneously and orally to goat No. 2 in Experiment No. 1 failed to produce the disease. The goats used

for this experiment, and experiments to follow were in lactation.

Preparation of inocula. Milk in all the experiments when given via the teat canal was diluted with boiled distilled water, in ratio of 3:1, after the milk had been filtered through sterilized gauze to free it from blood and fibrin clots.

TABLE III

Material obtained from donor goat No. D3

Expt. goat No.	Date	Route	Material	Dose	Remarks
8	April 4, 42.	Left teat canal	Milk	15 c.c.	Acute changes seen in left teat. Rt. teat remained normal. Changes in milk in both the udders resembled those seen natural cases. Died on 23rd April 1943

Thus a disease confined to the udder was produced in goat No. 8 (Table III).

Experiment No. 4

This experiment was designed to see if milk from exptl. goat No. 8 was infective. One healthy goat No. 9 was injected via the teat canal, the left and right teat canals receiving respectively 20 c.c. and 10 c.c. of milk from the left and right side of the udder of goat No. 8. Acute changes were seen in the left side of the udder, and the milk was greatly reduced in quantity. Eight days after the inoculation, acute lameness and swelling of the right knee joint were observed such as are seen in natural cases.

Thus the disease was successfully passed from one experimental animal to another, and typical symptoms of the disease were produced.

Experiment No. 5

One healthy goat No. 10 was housed and fed with donor goat No. D3 received from Jamesabad (from the 4th April to 23rd May 1942 when donor goat died) in order to see if the disease could be

conveyed by contact. The receptor, however, showed no inflammatory lesions of the udder or joints, and the temperature remained normal. The animal, however, showed changes in the milk and udder, became emaciated, and died on the 11th June 1942.

Thus the disease appears to be transmissible by contact, when the diseased and healthy goats are housed and fed together.

Experiment No. 6

Experiments No. 3 and 4 proved that the infective agent is present in the milk and that the disease could be conveyed via the teat canal. This experiment was designed to see if the disease could be produced in healthy lactating animals by injecting milk subcutaneously or intra muscularly. Inocula consisted of three collective samples of mammary exudate taken from three batches of four, three and three goats suffering from *Wah* received from Laso Mahomed village, Khipro and Mirpurkhas on 23rd October 1942, 22nd October 1942 and 1st February 1943, respectively.

TABLE IV

Expt. goat No.	Date	Route	Material	Dose	Remarks
11	29-10-42	Subcut.	Mammary exudate	10 c.c.	Only local reaction was produced at the site of injection. Goat was kept under observation for 1½ months, but showed changes in milk only
12	Do.	Intra-muscular	Do.	8 c.c.	Changes in milk were noticed on 9th day and inflammation of knee joint and lameness on 13th day after inoculation. Died on 26 November 1942. Typical disease was produced
13	9-2-43	Do.	Do.	10 c.c.	Acute inflammation at site of injection, goat went dead-lame and off-feed. In course of three days inflammation completely subsided. On 10th and 11th day after injection, changes in milk and the inflammation of knee were noticed. Typical disease was produced

The results of experiment No. 6 (Table IV) showed that the disease could be produced in susceptible animals by subcutaneous and intra muscular inoculation of milk from affected cases.

Experiment No. 7

This experiment was designed to see if the infective agent, which is believed to be a filterable

virus, present in the milk, can be destroyed by heat at 60° C. for half-an-hour, as most known viruses are destroyed at temperatures ranging between 58°C. and 60°C. This experiment also served to show whether infection could be conveyed from the experimentally-produced disease to a second healthy goat.

TABLE V

Expt. goat No.	Date	Route	Material	Dose	Remarks
14	27-2-43	Intra muscular	Mammary exudate from goat No. 13.	15 c.c.	Slight lameness in fore-legs and changes in milk. Subacute type of disease produced
15	1-3-43	Do.	"Do." heated 60°C. for $\frac{1}{2}$ an hour.	15 c.c.	No reaction

This experiment (Table V) shows that the disease can be passaged, and that the causative agent is destroyed when heated to 60°C. for half an hour.

DISCUSSION

According to the 1939-40 Annual Report of the Imperial Veterinary Research Institute, *Wah* was believed by early workers to be rinderpest, anthrax or haemorrhagic septicaemia. The present experimental and field investigation shows that *Wah* is none of the above-named diseases.

As already indicated, no growth has been obtained by sowing samples of morbid material on laboratory media, and no pathogenic organisms could be seen on microscopical examination of milk, blood or knee joint fluid. It seems that the causative agent will probably prove to be a filterable virus. (Attempts at isolating a virus could not be made in the author's laboratory owing to lack of facilities).

The chief symptoms of the disease, viz, abortion, swelling of the joints, lameness, disturbances of milk secretion and keratitis are also seen in goats and sheep suffering from *Brucella* infections [Dobois, 1926]. An examination of urine, milk, blood and blood sera from the affected cases, however, indicated that *Wah* is not due to *Brucella* infection, while the absence of undulant fever in human beings consuming the milk from affected goats further supports this conclusion. The symptoms of the disease, as seen in natural cases and as shown by experimental animals, resemble contagious agalactia of sheep and goats [Wooldridge, 1923; Galloway, 1930]. The writer has been able to reproduce the disease in healthy

animals by inoculation of altered milk, which is in conformity with the findings of Celli and De Blasi [1896]. After the introduction of virulent material, viz. mammary exudate, into the mammary gland, the milk was altered after three or four days; in some cases the infection remained localized, in others after 10 to 12 days there was generalization to the knee joints. This finding is in agreement with that of Galloway [1930], the only difference being that no eye lesions were shown in the writer's experimental animals. The infective agent, after heating to 60°C. for half-an-hour, failed to produce the disease in healthy susceptible animals. These results are also comparable with those of Galloway [1930].

The above evidence tends to show that *Wah* of goats and sheep, as found in Sind, bears a strong resemblance to contagious agalactia of sheep and goats as found in Italy [Metaxa, 1816] and Algeria [Sergent & Roig, 1917]. In the Encyclopedia of Wooldridge [1923] it is stated that the disease is limited to Europe, particularly the mountainous districts of Switzerland, Northern Italy and Southern France. This is the first record of the disease in India.

As to treatment, no specific curative agent has been found for this condition. Bridre, Donatien and Hilbert, [1928] in a small number of experiments obtained good results with stovarsol. This drug has not been tried by the writer owing to its present high price. Palliative treatment by oral administration of magnesium sulphate and salicylic acid has given apparently beneficial results. The intravenous injection of Lugol's solution and formalin has also been tried on a few animals with varying success.

SUMMARY AND CONCLUSIONS

1. The disease of goats and sheep known as 'wah' occurs as a specific epizootic in Sind and is transmissible experimentally.
2. The disease bears a strong resemblance to the condition known as 'contagious agalactia' and this is the first record of its occurrence in India.
3. The symptoms, course and pathology of the disease are described.
4. The pathogenic agent appears to be a filterable virus, since the disease is infectious and no specific pathogenic organism could be isolated from the lesions.
5. The causative agent is destroyed by heat at 60°C. for half-an-hour.
6. The incubation period in experimental goats is 5 to 11 days.
7. Inoculation of mammary exudate intramuscularly or by the teat-canal set up a disease similar to that seen in natural outbreaks.
8. A healthy goat subjected to close contact with a natural case developed the chronic form of the disease confined to the udder.
9. Lactating animals appear to be more susceptible.

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A CASE OF TUBERCULOSIS IN A SHEEP

By P. N. NANDA, M.R.C.V.S., Superintendent and KARNAIL SINGH, L.V.P., Government Cattle Farm, Hissar

(Received for publication on 28 August 1943)

THE incidence of tuberculosis in sheep is extremely low. Although rare cases have been recorded in a number of other countries, the present one appears to be the first case recorded in India.

SUBJECT

A female lamb No. 503 (*Lohi* breed) 15 months old.

HISTORY

None. The carcase was received for post-mortem examination in the Farm Veterinary Hospital.

POST-MORTEM FINDINGS

Nothing unusual was noticed from the external appearance of the carcase. On opening it pathological changes were noticed in the thoracic cavity. Both the lungs were adherent to the thoracic wall and the pleura at these places were thickened. The surface of the lungs showed greyish white patches varying in size from a pinhead to a walnut and giving them a mottled appearance. Still bigger patches of

irregular shape were seen as a result of coalescence of the smaller ones. The patches were level with the lung surface and did not show any elevation. The lung tissue on removal was found to be firm, enlarged and hepatized. On section through the patches, caseation with calcification was noticed. The substance of both the lungs was extensively involved and very little tissue was left unaffected. There was no evidence of any cavity formation. The associated lymph glands were enlarged and indurated. On cutting through these glands, caseation and calcification was found.

Nothing abnormal was found in the other organs.

LABORATORY FINDINGS

Smears from the lungs and affected lymph glands were prepared and stained by the Ziehl-Neelsen method. On examination under the microscope, acid fast organisms indistinguishable from tubercle bacilli were detected.

Material was sent to the Research Officer, Tuberculosis and Johne's Disease, Imperial

Veterinary Research Institute, Mukteswar, who reported: "The diagnosis of tuberculosis was confirmed and the strain of tubercle bacillus isolated proved to be of the bovine type".

A high incidence of tuberculosis was noticed in cattle on this Farm in 1939-40, though, at

present, it has been considerably reduced by judicious management and suitable preventive measures. It is highly probable that this sheep contracted infection from the bovine stock on the farm, particularly as the bovine strain of tubercle bacillus was responsible for the disease.

SOME OBSERVATIONS ON THE LIFE-HISTORY OF THE DOG TICK *RHIPICEPHALUS SANGUINEUS* (LATREILLE) AT MUKTESWAR*

By S. N. SAPRE, B.Sc., G.B.V.C., Research Scholar, Imperial Veterinary Research Institute, Mukteswar-Kumaun

(Received for publication on 1 December 1943)

The greater part of the material used in this work was received from Bombay and Baroda. The observations were made at Mukteswar (alt. 7,500 ft.).

R. Sanguineus does not appear to adapt itself as readily as *Boophilus australis* to the atmospheric conditions existing at Mukteswar, and specimens of this species could only be collected in very small numbers from local dogs.

Christophers [1907] made an extensive study on the life-history of *R. sanguineus* under Indian conditions but this was incidental to his work on the life-cycle of *Babesia canis* in this species of tick. It is evidently on this account that his records are not complete and do not indicate the temperature at which the specimens were reared. Nuttall [1914], at Cambridge, worked out the life-history of this parasite in some detail at 30°C. The present observations on the non-parasitic stages of the tick were carried out at 22°C., a temperature slightly lower than that used by Nuttall, and a relative humidity of 80 to 90 per cent. In all cases the parasitic stages of the tick were accomplished on the dog.

I. *Observations relating to oviposition.* Two batches, the first of 29 and the second of 20 gravid females, were kept under observation. Oviposition commenced three to six days (av., four days) after the gravid female left the host, the process of oviposition lasting 14 to 18 days (av., 15.6 days). The female survives 2 to 14 days (av., six days) after oviposition ceases and lays 1485 to 3556 (av., 2140) eggs.

II. *The duration of egg stage.* The duration of the egg stage as calculated from the commencement of oviposition to the date of emergence of the first larva was found to be from 25

to 40 days (av., of first batch 31.9 and of the second batch 31.3 days).

III. *The larval stage.* More than a 1,000 individuals were observed. These divided into 16 batches were put on the host for feed, the host being maintained at 1.4 to 11.6°C. The percentage of engorged larvae recovered on the fourth day was 62 to 75, on the fifth day 14 to 30 and on the sixth day 1 to 2 only. In one instance alone engorged larvae were recovered on the third day, and these were very few. The non-parasitic period of the larval stage was 24 to 30 days at 22°C.

IV. *The nymphal stage.* About 325 individuals were divided into six batches. The temperature at which the host was maintained was 2.9 to 5.1°C. The percentage of engorged nymphs recovered on the fifth day was negligible, that on the sixth was 40 to 54.5, on the seventh 28 to 45, on the eighth 5 to 9.5 and on the ninth 1.2 to 6.3. The non-parasitic period of the nymphal stage varied from 30 to 43 days. Of the 325 nymphs 193 emerged as females while 129 as males. The sex ratio was approximately 2 males to 3 females.

V. *The adult stage.* 49 females and 32 males in two batches were put on the host to feed. The host was kept at 11.0 to 14.6°C. Daily recoveries of replete females were 9, 22, 6, 6, 4, 1, 1, from the 9th to 15th day.

SUMMARY

R. Sanguineus requires three hosts upon which to feed. At 22°C. the oviposition lasted for 15 days, the egg stage was 31 days, parasitic periods for larvae, nymphs and adults were 4, 61 and 10 days and the non-parasitic periods were 27, 36 and 25 days respectively. The parasitic periods of the parasite remained constant irrespective of the variations in tempe-

*Paper read at the Indian Science Congress held at Baroda, January 1942.

perature, on the other hand the non-parasitic periods appear to be inversely proportional to an increase or decrease in temperature.

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POISONING OF LIVESTOCK BY *DATURA STRAMONIUM L.*

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SUDDEN illness and death occurring among draught animals at pastures on the Institute land, Mukteswar, led us to suspect the ingestion of some poisonous plants. Consequently, a survey of the toxic plants of that area was initiated and feeding experiments with some of the plants were carried out. This article in-

cludes observations on the feeding of *Datura stramonium* to farm animals.

The tender parts of the plant, consisting of the leaves, flowers, fruit and the soft stalk, such as are ordinarily eaten by stock in grazing, were chopped and fed to two bulls, two sheep and two goats (Table I) after a preliminary fast of 24 hours.

TABLE I
Mukteswar variety

Dates	Species of animals	No.	Material fed	Method of feeding	Quantity fed during experimental period (lb.)	Symptoms	Remarks
22-7-38 to 30-7-38	Kummauni bulls	2	Whole plant chopped	Voluntarily. In green grass or concentrate mixture	16-17	Nil	<i>Datura</i> , whole plant in the unripe capsule stage
24-10-38 to 28-10-38	Do.	..	Whole chopped plant with greens	Voluntarily	8-9	Nil	<i>Datura</i> , in the ripe and unripe stage
29-10-38 to 2-11-38	Do.	..	Crushed plant suspension in water	Drenched	10	Nil	Do.
3-11-38 to 11-11-38	Ripe and unripe seeds crushed in water as suspension	Do.	2	Nil	Do.
22-7-38 to 30-7-38	Goats	2	Whole plant chopped	Voluntarily. In green grass or concentrate mixture	9-10	Nil	<i>Datura</i> , whole plant in the unripe capsule stage
24-10-38 to 28-10-38	Sheep	2	Do.	Do.	8-9	Nil	Do.
	Goats	2	Do.	Voluntarily	4-4½	Nil	<i>Datura</i> , in the ripe and unripe stage
29-10-38 to 2-11-38	Sheep	2	Do.	Do.	4	Nil	Do.
	Goats	2	Crushed whole plant suspension	Drenched	2½	Nil	Do.
3-11-38 to 11-11-38	Sheep	2	Do.	Do.	2½	Nil	Do.
	Goats	2	Ripe and unripe crushed.	Do.	1	Nil	Do.
	Sheep	2	Seed suspension in water.	Do.	1	Nil	Do.

The chopped plant was mixed with concentrates or green fodder. For the first eight days the animals took the chopped plant voluntarily. Subsequently, as no ill effect was observed, the feeding was continued by drenching the plant suspension in water. Even then, however, the animals showed no symptoms of toxicity.

In view of the numerous cases of accidental and homicidal poisoning with the plant, reviewed by Van Itallie and Bylsma [1928] and Steyn [1934], it was considered that the soil and the

climatic conditions in the hills at a height of 7,500 ft. might have affected the alkaloidal content of the plant.

A feeding experiment similar to the above was, therefore, undertaken at Izatnagar (Table II) in the plains. The few symptoms that the bulls showed on feeding at the rate of about 1-2 lb. per day were constipation, bulging of the eye-balls with reddish discolouration of the lens, dilation of the pupils, slight drowsiness and an accelerated pulse.

TABLE II
Izatnagar variety

Dates	Species of animals	No.	Material fed	Method of feeding	Quantity fed (lb.)	Symptoms	Remarks
22-10-42	Kumauni bull Wt. 152 lb.	1	Whole chopped plant with concentrate 9 a.m.	Voluntarily	3	12-30 Droopy falling head. 2-30 eyes discoloured, bulged. Quick pulse. 23/10 faeces constipated	Datura, in ripe and unripe capsule stage
Do.	Kumauni bull Wt. 224 lb.	1	Do.	Do.	Do.	Nil	
23-10-42	Do.	..	Do.	Do.	2 each	Faeces constipated in both at 2-30; in one eyes discoloured and reddish, bulged. 24/10. In the other bull, drowsiness at 3 P.M.	
24-10-42	Do.	..	Do.	Do.	
25-10-42 to 28-10-42	Whole ground plant	As bolus by hand	9 each	Nil	
24-10-42 to 27-10-42	Goat	2	Whole chopped plant in concentrate	Voluntarily	3-3½ each	Nil	
24-10-42	Sheep	2	Do.	Do.	2½ each	Nil	
25-10-42	Do.	..	Whole ground plant	As bolus by hand	2½ each	Nil	
26-10-42 to 27-10-42	Do.	Do.	Do.	Do.	Do.	Do.	

These observations are in conformity with those of Steyn [1931], though Frohner [1919] reported the death of a horse within 52 hours of feeding 2.25 kg. of mature seeds.

ACTIVE PRINCIPLE

Van Itallie and Bylsma [1928] found the total alkaloidal content of 0.2 to 0.5 per cent to consist chiefly of hyoscyamine in the leaves and 0.2 to 0.4 per cent of hyoscyamine and hyoscyne respectively in the seeds; Steyn [1934] reported a total alkaloidal content of 0.43 per cent, chiefly hyoscyamine. Chou [1935] discovered datugen and datuginine amounting to 0.3 per cent., in addition to hyoscyamine, hyoscyne and atropine. Fluck [1939] found that re-wetting or checking the drying process and harvesting the wet plant gave low alkaloidal yields. Sirgo [1939] observed that *Datura* seeds exposed to acetylene or carbon monoxide gases matured earlier but contained a lower alkaloid content than normally-ripened seeds.

DISCUSSION

The observations recorded in Table I and II indicate the innocuous nature of *Datura stramonium* growing in the two localities. A number of factors affect the toxicity of the plant, important among them being (1) the amount eaten in a given period of time; (2) the condition of the soil—its porosity, moisture and oxygen content; (3) climatic conditions, which may be responsible for the production of a variable quantity of the alkaloid by their direct action on the microbiological processes, which in their turn may affect the toxicity of the plant; (4) cultivation, which not only decreases the toxicity but in some cases, as in gourds, causes the entire toxicity to be lost [Suyi Chen and Wei Jenkao, 1936], while the difference in toxicity between the so-called wild plants of one region and another may be due to their being recent escapes from cultivation [Watts, 1908]; (5) the time of

year, and even the time of day, which may influence the toxicity of a plant, [Miklos Jamesek, 1932]; (6) the distribution of the toxic principle, which may not be the same in all parts of the plant; (7) the transmission of toxicity to progeny as seen in different degrees in *Atropa belladonna* and other plants; (8) the stage of growth of the plant and (9) the nature and intensity of light, which influences the alkaloid content of *Datura* from 0.1 to 0.254 per cent [Werner Braun, 1939].

It seems that the alkaloidal content in these plants was not high enough to cause typical toxic symptoms. However, from some of the mild symptoms observed by feeding the plant at Izatnagar it appears that the hill variety is even poorer in these alkaloids.

SUMMARY

Two samples of *Datura stramonium* Lin, a hill variety and a plains variety, were fed to bulls, sheep and goats, to study their toxicity. The hill variety appeared to be considerably poorer in alkaloid content than the plains variety. Both the plants were innocuous.

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NOTE ON THE METHOD OF CALCULATING SIRE INDEX FOR MILK PRODUCTION IN CATTLE

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There are two ways of using the information about the progeny of a sire in judging his worth: Progeny Test and Sire Index. The former consists in making a test of significance of the

difference in yields between the daughters of a sire and their mothers and shows whether a sire can produce daughters which average better in milk yield than their dams, when the dams

are of the kind available on the farm. It does not show how the sire would behave if the dams are of a higher level, nor does it help a breeder to compare the breeding value of two bulls from different forms. The Sire Index, on the other hand, is an estimate of the breeding value of a sire in terms of yield of milk and can tell a breeder what and how much he can expect of a sire when used on mates of any known level.

It follows that a breeder would prefer to adopt a sire index rather than a progeny test, provided the sire index gave a reliable estimate of the breeding worth of sire. In actual fact it is doubtful if sire index is absolutely so. The common form of sire index, known as the 'Commercial Mount Hope Index', makes use of the assumption that the offspring averages midway between the two parents. We do not know how far this assumption is valid, although in a character like the milk-yield which is determined by multiple genes, it is not expected that this assumption would be far from the truth. Then again a sire index is relatively more influenced by environmental differences than is a progeny test. As against these considerations progeny test in Fisher's 't' form, as observed above, is of limited use to a breeder. Logically the progeny test and sire index are not mutually exclusive, as one is a test of significance and the other is a problem in estimation, and, therefore, the question of preferring one to the other does not arise. The appropriate course is to make a test of significance and then construct a sire index. This, however, would involve considerable computational work. For the present, therefore, it is suggested that the use of sire index alone be introduced in our breeding farms.

It is not proposed to discuss the relative merits of the various indices that have been advocated for measuring the breeding worth of a sire. The most common of these are (1) the Daughter Average Index, which is the average milk-yield of all daughters of a sire, and (2) the Commercial Mount Hope Index, also known as the Intermediate Index, which is the difference between twice the average milk-yield of daughters and the average milk-yield of their mothers. The first uses information on all the daughters of a sire, regardless of whether their mothers were tested or not, and consequently, is suitable for application in villages and on new farms where the mothers' milk-yield may not be known, whereas the second uses information in respect of only such daughters, whose mothers have been tested. The first is a statement of fact independent of any assumption, whereas

the second is a hypothetical figure based on the assumption that the offspring average midway between the parents and is, therefore, dependent for its reliability on the validity of this assumption. Both are affected by environmental errors, but the second probably is more affected than the first. Notwithstanding these advantages in favour of the first, the Commercial Mount Hope Index is generally preferred by the breeders, because it helps to predict the performance of a sire on mates of any known level, whereas the other, does not. Further statistical research on Indian data on the relative efficiency of the two indices, and on the validity of the assumption used in the construction of the second index, is clearly needed, but in the meantime, it would appear that the Intermediate Index would be the more useful.

The formula for the Intermediate Index is directly derived from the assumption that the offspring averages midway between the sire and the mother, and is given by

$$I = 2D - M$$

where D and M are the average milk-yields of daughters of the sire and his mates (mothers of daughters) respectively. The reliability of this index is obviously dependent on the magnitude of the environmental and the sampling errors. The former of these errors arise from the difference between the environments of mothers and daughters and the possible selection of mothers, whereas the latter, arise principally from the sampling nature of inheritance. Where it is reasonably certain that the environmental conditions of daughters are not distinct from those of their mothers, lest we credit or blame the sire for something which was due to the environment and where the mothers have not been particularly selected, this index is influenced only by the errors of sampling and can be depended upon to give a reliable idea about the sire—provided a sufficiently large number of offspring is available. But environmental conditions usually differ not only from farm to farm, but also between dams and daughters on the same farm. It is, therefore, necessary to equalize the environmental conditions, so far as is feasible, so as to reduce to the minimum the environmental errors of the index. By omitting locations which are abnormal on account of either (1) death of calf or still-birth, or (2) diseased condition of mother or daughter or (3) any other abnormal factor, a large degree of equality in the environmental conditions can be secured. At the same time it is also necessary to standardize other major

factors affecting milk-yield in order to reduce sufficiently the environmental errors. This is usually done by standardizing (1) the order of location, (2) the length of lactation and (3) the number of milkings per day.

The effect of selection of dams is to underestimate the value of the sire i.e., to make the sire appear as if he does not possess a high measure of the transmitting ability, when in fact he does. The means by which we can allow for this effect, at least partially, is to use a slightly larger level of probability in tests of significance than is conventionally done. This will be explained later.

It is usual to include first lactation yields in calculating the index even though they are not an absolute index to the performance in later lactations. The reason for their use is that a breeder wants to have the earliest indication regarding the value of a sire and cannot wait until all the later lactation yields are available. It is, of course, advisable to recalculate the index, as later lactations are recorded, but care should be taken to avoid the bias that is likely to be introduced by the selection of dams and daughters that may have taken place in the meantime. Usually, the later lactation yields are reduced to their equivalent first lactation yields with the help of approved conversion factors before recalculating the index. No such factors have, however, been worked out for use in India. The appropriate course is to work out a regression of yield on the order of lactation from the material locally available and use it to convert the later lactation yields to their equivalent first lactation yields before recalculating the index. This would secure the comparability of one index figure with another. The procedure can be used whether the lactations of dams and daughters are the corresponding lactations or not. Where the order of lactation of all mates of a sire is not known, though records of milk-yields are available, the procedure is only slightly different. No standard method can be suggested in either case, but statistical technique to suit the requirements of the data can be devised.

Length of lactation is another important factor influencing the yield of milk. If the lengths of lactation of dams and daughters are unequal, sire index based on lactation yields may give a misleading idea about the value of the sire. Table VI showing the dam-daughter differences for yield and the length of the first lactation, gives an idea of the extent to which the length of lactation may influence the yield of milk. In calculating the index, it is usual to allow for the difference in the length

of lactation by considering the milk-yields of the first 300 days. In India just now, it might be possible to provide for the reporting of the first 300 days' yield in registers maintained on some farms, but it will be sometime before this can become general. In the meantime, the only course available is to recalculate the yields from what records there are, but these may not suffice; and at any rate, the procedure would involve considerable time and labour. As an alternative, the lactation yields may be adjusted to the standard length of 300 days by the use of regression. Logically, this is actually a more appropriate method, since it uses complete information on lactation yields. Moreover, it has an added advantage of minimizing the sampling error of the sire index. In this method, the observed relationship between the yield and the length of lactation is used to adjust the yields to the standard length of lactation. Details of the method are illustrated later in Tables II and III.

The number of milkings per day is another factor influencing the yield of milk. Milk-yields obtained under twice-a-day milking conditions are usually used in calculating the index. Where the number of milkings is more than two, the records are usually corrected to twice-a-day milking condition with the help of approved conversion factors. In India, twice-a-day milking is a general, and consequently, the use of such factors would be only rarely called for in practice. Where, however, they are needed, they may be worked out from the available past records.

Even when the milk-yield records of dams and daughters are equalized, the differences between them will still be affected by errors which are partly systematic and partly random. In so far as the systematic errors are concerned, one can merely make a close study of the environment and make allowance such as one thinks to be the fairest for departure from normal conditions, but beyond that, there is little that one can do, e.g., differences arising from the practice of weaning in mothers and daughters. Random errors are easier to deal with. They cancel out in the process of averaging. Consequently, when the number of dam-daughters pairs is sufficiently large, the sampling errors of the average milk-yields is small, and the index can be relied upon to give an accurate indication of the breeding worth of a sire.

The concept of sampling error is relatively new to breeders, consequently, it is not yet usual to calculate the sampling error as a guide to the reliability of the index. It is, however,

obviously important not to misjudge the value of a sire by taking his calculated index at its face value. The calculated index is only an estimate in terms of yield of milk of the true breeding worth for milk production of a sire. It is, therefore, imperative to know the magnitude of the sampling error, so that a breeder may know the extent to which the index is likely to vary from chance causes.

The sampling error is measured by what is known as the standard error in statistics, and is calculated in the manner illustrated in the examples given below. It is usual to add and to subtract from the calculated value of the index a fixed multiple of the standard error to know the extent to which the index is likely to vary due to chance. This multiple is given by

the value of Fisher's t corresponding to the number of pairs less one or used in calculating the index. By convention Fisher's t at the 5 per cent. level of probability is used as multiple. In breeding problems, however, it seems appropriate to use the 10 per cent level of probability since by continuous selection the genetic variance is reduced to a level at which genetic differences become difficult to detect. It is as important not to understate the value of a sire as it is important not to overestimate it; and it would, therefore, seem that 10 per cent is the most suitable level. The table below shows the 10 per cent values of t taken from Fisher's book on 'Statistical Methods for Research Workers' and is reproduced for ready use by breeders.

$n =$	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10% =	6.314	2.920	2.353	2.132	2.015	1.943	1.895	1.860	1.833	1.812	1.796	1.782	1.771	1.761	1.753
$n =$	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
10% =	1.746	1.740	1.734	1.729	1.725	1.721	1.717	1.714	1.711	1.708	1.706	1.703	1.701	1.699	1.697

As to how many dam-daughter pairs are necessary to prove a sire, the answer depends upon how sure of his proof one wants to be and how much can be gained by testing more daughters. If the systematic differences between the environments of dams and daughters are large, even after carrying out all the suggested adjustments, the accuracy of the sire index will remain low under any circumstances howsoever large the number of dam-daughter pairs may be. On the other hand, if the environmental differences between dams and daughters are correctly discounted, the sire index may be considerably increased in accuracy by increasing the number of dam-daughter pairs. It will thus be seen that it is difficult to fix the minimum number of dam-daughters pairs required for calculating the index. The number of dam-daughter pairs as recommended by different workers varies widely. While there is no harm in using as low a number of pairs as two or three for deriving an indication of the breeding worth of a sire, it seems undesirable to publish an index based on fewer than six pairs.

The concept of sampling error is applicable to a group of records which can be adequately expressed by means of their average and the degree of variability around it. When we say that dams on a farm are of a particular level, we imply this condition. Consequently, it is important to make a statistical test of significance to cull out extreme records which cannot be regarded without hesitation, as random members of the group. Such a test is valid only after the records are adjusted to environmental differences and is justified only on the mothers' records since an exceptional record in daughters may result from the sampling nature of inheritance. It is, however, beyond the scope of this note to describe this test for the rejection of extreme records.

The detailed procedure of calculating the Intermediate Index is explained below on the records of Sindhi bull No. 98 from Hosur Cattle Farm, Madras. Table I shows the main steps involved in the calculations of the index. Columns 2, 3, 4 and 5 of the table show the milk yield records with the corresponding num-

Sire Index for Milk Production in Cattle

ber of days of the first lactation of mothers and daughters—sired by the bull. Of these, the lactation records of daughters of the 5th, 9th and the 16th pairs and of the mother of the 8th pair are abnormal for reasons mentioned in the remarks column, and are, therefore, omitted from the calculations. In the remaining records of mothers, there is none that is, either exceptionally high, or low, which can be regarded as incompatible with the homogeneity of the group. Columns 6 and 7 show the yields of mothers and daughters adjusted to the standard length of 900 days by the methods illustrated in Tables II and III. Column 8 shows the differences between twice the adjusted yield of daughters and the adjusted yield of mothers. Column 9 shows the squares of the figures in col. 8. The mean value of figures of column 8 is the Intermediate Index adjusted to the standard length of 300 days. In practice, it is not necessary to carry out the calculations to the

last unit as shown in the table. The calculations can be simplified by rounding the figures in the 8th column to the nearest tens.

From the sum of the 9th column is subtracted a figure, known as the correction factor for the sum of squares, which is equal to the quotient of the square of the total of column 8 by the number of pairs used. The figure so derived is called the corrected sum of squares. The corrected sum of the squares divided by the product of the number of pairs used with the number of these pairs less one is the sampling variance of the Intermediate Index. The square root of the sampling variance is the standard error of the index. As the 10 percent value of t corresponding to the number of pairs less one is 1.796, the extent to which the calculated index is likely to vary on either side of its value is given by the product of the standard error and 1.796 and is shown in the last row of the table under column 9.

TABLE I

*Showing the calculation of the Intermediate Index for milk production of Sindhi Bull No. 38 from
Hosur Cattle Farm, Madras*

No.	Milk yield in lb.		Days of Lactation		Adjusted yields in lb.		Twice col. 7 minus col. 6	Square of col. 8	Remarks
	Mothers	Daughters	Mothers	Daughters	Mothers (from Table II)	Daughters (from Table III)			
1	2	3	4	5	6	7	8	9	10
1	2631	3090	346	262	1872	3711	5550	30802500	
2	3454	3149	349	329	2646	2720	2794	7806436	
3	2034	2941	271	280	2612	3268	4024	16192576	
4	3760	3645	376	372	2666	2468	2430	5904900	
5	4191	931	343	87*	* Still Birth
6	3537	4967	324	373	3141	3774	4407	19421649	
7	4810	5241	385	385	3408	3851	4294	18438436	
8	1902	5290	173†	372	† Calf Death
9	3217	854	332	127†	† Calf Death
10	4115	4474	349	361	3307	3477	3647	13300609	
11	4297	4904	391	353	2796	4038	5280	27878400	
12	3765	5313	339	374	3122	4103	5084	25847056	
13	4972	4158	340	317	4312	3880	3448	11888704	
14	3662	4966	374	352	2441	3625	4809	23126481	
15	3556	3833	362	293	2563	3952	5341	28526281	
16	6943	1492	384	207†	† Still birth
Total	44623	50731	4206	4081	34626	42867	51108	229134028	
Average	3718.6	4227.6	350.5	340.1	2885.5	3572.2	4269	..	

Correction factor for sum of squares	(51108)	217668972.00
	12	
Corrected sum of squares		11465056.00
Sampling variance of intermediate index		86856.48
Standard error of intermediate index		294.7 lb.
Standard error $\times t$ 10 %		529 lb.

It will be seen that the value of the Intermediate Index is 4259 lbs., its sampling error is 295 lb. and the extent to which the figure of 4259 is likely to vary due to chance is 529. In other words the best estimate of the true breeding worth of the sire measured in terms of yield of milk is 4259, but this estimate is likely to err on either side to the extent of 529. A breeder thus knows that, provided the environmental errors are correctly discounted, he can use the sire with confidence for increasing production of daughters when the mates are of a level below 8730 lb.

Tables II and III give the calculations required for adjusting the yields of mothers and daughters to the standard length of 300 days. From the total of the figures in col. 4 is subtracted a figure called the correction factor,

which is equal to the quotient of the square of the total of col. 3 by the number of pairs. The figure so derived is the corrected sum of squares of the days of lactation. Similarly from the total of the figures in col. 5 is subtracted a figure, a correction factor, which is obtained by dividing the product of the totals of col. 2 and 3 by the number of pairs. The remainder is called the corrected sum of products. The corrected sum of products divided by the corrected sum of squares of the days of lactation gives a figure called the regression coefficient, 'b', of the milk yield on the days of lactation. In column 6 are entered 300 *minus* the figures in col. 3. Column 7 shows the products of the figures in col. 6 with 'b'. Column 8 gives the sum of the corresponding figures in cols. 2 and 7 and shows the yields adjusted to 300 days of lactation.

TABLE II

Showing the method of adjusting yields of mothers to the standard length of 300 days

No.	Yield in lb.	Days of lactation	Square of col. 3	Product of col. 2 and col. 3	300—col. 3	Col. 6 × 'b' = Col. 6 × 16·501	Col. 2 + Col. 7
1	2	3	4	5	6	7	8
1	2631	346	119716	910326	— 46	— 759	1872
2	3454	349	121801	1205446	— 49	— 808	2646
3	2034	271	73441	551214	29	478	2512
4	3760	376	141376	1413760	— 76	—1254	2506
5	3537	324	104976	1145998	— 24	— 396	3141
6	4810	385	148225	1861850	— 85	—1402	3408
7	4115	340	121801	1436135	— 40	— 808	3307
8	4297	391	152881	1680127	— 91	—1501	2796
9	3765	339	114921	1276335	— 39	— 643	3122
10	4972	340	115600	1690480	— 40	— 660	4312
11	3662	374	139876	1369588	— 74	—1221	2441
12	3586	362	131044	1298132	— 62	—1023	2563
Total	44623	4206	1485658	15829381	—606	— 9997	34026

Correction factor	$(4206)^2$	1474203	44623×4206
	12		12
Corrected total	..	11455	$= 15640361 \cdot 5$
	189019·5
Regression coefficient of milk yield on days = 'b'	189019·5
			11455·0
			= 16·501

TABLE III

Showing the method of adjusting yields of daughters to the standard length of 300 days

No.	Yield in lb.	Days of lactation	Square of col. 3	Product of col. 2 and col. 3	300 - col. 3	Col. 6 \times 'b' = Col. 6 \times 16.350	Col. 2 \div Col. 7
1	2	3	4	5	6	7	8
1	3090	262	68644	809580	+38	+ 621	3711
2	3194	329	108241	1050826	-29	- 474	2720
3	2941	280	78400	823480	+20	+ 327	3268
4	3645	372	138384	1355940	-72	- 1177	2468
5	4967	373	139129	1852091	-73	- 1193	3774
6	5241	385	148225	2017785	-85	- 1390	3851
7	4474	361	130321	1615114	-61	- 997	3477
8	4904	353	124609	1731112	-53	- 866	4038
9	5313	374	139876	1987002	-74	- 1210	4103
10	4158	317	100489	1318086	-17	- 278	3880
11	4966	382	145924	1897012	-82	- 1341	3625
12	3838	293	85849	1124534	+7	+ 114	3952
Total	50731	4081	1408091	17583222	-481	- 7864	42867

		50731	\times	4081
	(4081) ²			12
	12			
Correction factor		1387880.08	=	17252767.58
Corrected total	..	20210.92		330454.42
Regression coefficient of milk yield on days='b'	..	330454.42		16.350
		20210.92		

Two more illustrations of the procedure of calculating the Intermediate Index are given in Tables IV and V. Table IV shows the records of the main calculations for bull No. 8 from Hosur Cattle Farm, Madras; Table V for bull No. 56 from the same farm. All the records in the tables relate to the first lactation yields obtained under twice-a-day milking condition. The dam-daughter pairs Nos. 26 and 27 in Table IV and No. 7 in Table V are omitted from the calculation for reasons mentioned in the remarks column.

It will be seen from the table that details of calculating the Intermediate Index are identical with those presented in Table I. There are, however, two features, one in the records of Table IV and one in Table V, which require an explanation. A reference to col. 2 of Table IV shows that there are 21 different records of mothers corresponding to 21 different mates of the bull. The regression of milk-yield on the length of lactation is consequently based on 21 pairs of observations and not 25. The feature of Table V is the exceptionally high yield record

of the daughter of dam-daughter pair No. 4. The test of significance, however, shows that the record is not so high that it cannot be re-

garded as the random member of the group arising from the sampling nature of inheritance. Its inclusion in the index is therefore justified.

TABLE IV

Showing the calculation of the Intermediate Index for milk production of Sindhi Bull No. 8 from Hosur Cattle Farm, Madras

No.	Milk yield in lb.		Days of lactation		Adjusted yields in lb.		Twice col. 7 minus col. 6	Square of col. 8	Remarks
	Mothers	Daughters	Mothers	Daughters	Mothers	Daughters			
1	2	3	4	5	6	7	8	9	10
1	3042	2502	279	227	3405	3794	4183	17497480	
2	3282*	6152	293	368	3403	5032	6661	44368921	
3	4972	2409	340	365	4280	1338	1604	2572816	
4	3020	4678	288	383	3228	3311	3394	11519236	
5	3282*	3598	293	375	3403	2293	1183	1399489	
6	5103	5843	331	384	4567	3960	3353	11242609	
7	2004*	2847	290	331	2177	2336	2495	6225025	
8	1888	4176	167	351	4190	3336	2482	6100324	
9	3695	4403	346	364	2799	3429	4059	16475481	
10	7117	7774	402	437	5352	5518	5684	32307856	
11	4592	3997	338	344	3934	3272	2610	6812100	
12	4603*	7509	370	442	3452	5170	6888	47444544	
13	3052	5139	381	383	1650	3772	5894	34739236	
14	2004*	4015	290	297	2177	4064	5951	35414401	
15	3210	4351	312	382	3002	3000	2998	8988004	
16	3217	4868	332	327	2663	4423	6183	38229489	
17	2828*	3455	304	342	2759	2763	2767	7656289	
18	4663*	4934	370	365	3452	3363	4274	18267076	
19	5613	2266	344	265	4852	2842	832	692224	
20	2363	4531	315	360	2103	3543	4983	24830289	
21	3128	4459	329	359	2626	3487	4348	18905104	
22	4297	3167	391	217	2722	4534	6346	40271716	
23	3537	3879	324	377	3122	2611	2100	4410000	
24	2828*	3133	304	213	2759	4566	6373	40615129	
25	4200	4924	344	337	3439	4315	5191	26946481	
26	727**	4204	140	344	** Calf death † Still birth
27	416†	4754	104	386	
Total	91500	108609	8077	8595	81516	90572	99628	503901328	
Average	3660.0	4344.4	323.1	343.8	3260.6	3622.9	3985	..	
Correction factor for sum of squares							(99628) ² 25	397029535.36	
Corrected sum of squares								106961792.64	
Sampling variance of Int. Index								178269.65	
Standard Error of Int. Index								422.2 lb.	
Standard Error \times \pm 10 %								721 lb.	

NOTE.—Figures with asterisks * each appear twice in Col. 2

Sire Index for Milk Production in Cattle

TABLE V

Showing the calculation of the Intermediate Index for milk production of Sindhi Bull No. 56 from Hosur Cattle Farm, Madras

No.	Milk yield in lb.		Days of lactation		Adjusted yields in lb.		Twice col. 7 minus col. 6	Square of col. 8	Remarks
	Mothers	Daughters	Mothers	Daughters	Mothers	Daughters			
1	2	3	4	5	6	7	8	9	10
1	5103	7824	331	414	4780	4214	3648	13307904	
2	3874	4589	315	364	3718	2562	1406	1976836	
3	4339	5136	353	404	3788	1842	104	10816	
4	4376	10081	350	373	3856	7769	11682	136469124	
5	2878	3268	216	297	3752	3363	2974	8844676	
6	3595	5044	346	332	3116	4031	4946	24462916	
7	5241	1256*	385	217	*Sick
Total	24165	35942	1911	2184	23010	23781	24552	185072272	
Average	4027.5	5990.3	318.5	364.0	3835.0	3963.5	4092		
Correction Factor							(24552) ² 6	100466784	
Corrected sum of squares								84605488	
Sampling Variance of Int. Index								2820182.03	
Standard Error of Int. Index								1679.3 lb.	
Standard Error $\times 10\%$								2873 lb.	

Table VI gives a summary of the results relating to all the three bulls. It shows the average total milk yield of dams and daughters, the corresponding average lengths of lactation, the adjusted yields and the values of the Intermediate Index with their sampling errors. It shows at a glance the extent to which the difference in milk yield between dams and daughters is affected by the difference in the average lengths of their lactations. Thus, it will be seen that the increased production of daughters of bull No. 8 and 56 is in a large degree due to the extended length of lactation of daughters and not wholly to the transmitting ability of the bulls. The correct appraisal of the worth of the bulls is obtained when the records are adjusted to a standard length as shown in rows 8 and 9 of the table. The adjusted records clearly show that bull No. 8 is not such a transmitter as he appeared from the unadjusted milk yield records; bull No. 56 has hardly made any difference to the milk production of the daughters, and bull No. 38 is worth more than he looked from the unadjusted milk yield records. The correct value of the breeding worth of the three sires is shown by the Intermediate Index given in row No. 10.

TABLE VI

Showing the average total milk yields of mothers and daughters, the corresponding average lengths of lactation, the adjusted milk yields and values of the Intermediate Index with the sampling errors

	Sindhi Bull No. 38	Sindhi Bull No. 8	Sindhi Bull No. 56
1 Number of pairs	12	25	6
2 Average total milk yield of mothers (lb.)	3718.0	3860.0	4027.5
3 Average total milk yield of daughters (lb.)	4227.6	4344.4	5990.3
4 Average difference in total milk yield (daughter minus mother) (lb.)	509.0	684.4	1962.8
5 Average length of lactation of mothers (days)	350.5	323.1	318.5
6 Average length of lactation of daughters (days)	340.1	343.8	364.0
7 Average difference in length of lactation (daughter minus mother) (days)	-10.4	20.7	45.5
8 Average total milk yield of mothers adjusted to 300 days (lb.)	2835.5	3200.6	3835.0
9 Average total milk yield of daughters adjusted to 300 days (lb.)	3573.2	3622.0	5908.5

TABLE VI—contd.

	Sindhi Bull No. 33	Sindhi Bull No. 8	Sindhi Bull No. 56
10 Adjusted Intermediate Index (lb.)	4250	3985	4092
11 Standard error of Intermediate Index (lb.)	294.7	422.2	1679.3
12 Standard error $\times t$ 10 per cent (lb.)	520	732	2873

The table brings out very clearly the importance of calculating the sampling error of the index. It will be seen that although the three sires are of about the same value as judged by the calculated values of the Intermediate Index, the degree of reliance that can be placed on their breeding values is very different, e.g., it

would be wrong to recommend the use of bull No. 56 for increased production without additional proof of his ability, since on his present showing his actual worth may be as low as 1280 lb. On the other hand, it is almost certain that bulls Nos. 8 and 36 are worth more than 3281 and 3723 lb. of milk and can, therefore, be used with confidence on dams of those levels for increasing production.

ACKNOWLEDGEMENT

I am grateful to Mr N. R. Joshi with whom practically every view point expressed in this note was discussed and to Mr A. N. Sankaran for his help in the computations.

SOME NOTES ON THE LIFE-HISTORY OF *MUSCA PLANICEPS* WEID

By R. SENIOR-WHITE, F.R.S.E., Major, A.I.R.O. (Med.), Malariaologist, B. N. Railway

(Received for publication on 6 January 1944)

THE observations forming this note were made in the Ceylon foot-hills, 1,300 ft., 22 years ago. As the writer sees no chance of ever continuing them, they are now put on record, in the hope that they may be continued by another hand, as they show that the bionomics of this fly contain many interesting points.

The genus *Musca* is biologically divided into three groups, the saprophages, the haematophages, and the haematophiles. The latter obtain their supply of blood at punctures made by other biting flies, principally *Stomoxys* and the haematophages in the same genus. *M. planiceps* belongs to the haematophages.

M. cingalaisina Big. and *M. indica* Awati are synonyms of the species.

For eight months fresh cow-dung was exposed fortnightly for 24 hours and then removed to the laboratory for breeding out.

The emergences were:

1. <i>Hippelates</i> sp.	1 ♂	July
2. <i>Sepsis</i> sp.	7 ♀	September
3. <i>Sepsis rufa</i> Meig	1 ♀	July
4. <i>Brachyophyra</i> sp.	3 ♂ 2 ♀	July
5. <i>Spilogaster</i> sp.	9 ♂ 7 ♀	Every month except October and December
6. <i>Morellia hortensis</i> Wd. 34 ♂ 86 ♀		June and July
7. <i>Musca pattoni</i> Aust. . . .	4 ♂ 11 ♀	July, August, September, and November
8. <i>Musca bezzii</i> P. & C. . . .	2 ♂ 2 ♀	September and January
9. <i>Musca conducens</i> 2524 ♂ 2851 ♀		In every month Wk.

10. <i>Musca planiceps</i> Wd. . . .	57 ♂ 61 ♀	In every month.
11. <i>Musca inferior</i> Stein . . .	1 ♀	July
12. <i>Musca crassirostris</i> Stein .	34 ♂ 58 ♀	August, September, November, December
13. <i>Orthellia taota</i> Wd. . . .	8 ♂ 4 ♀	July
14. <i>Orthellia</i> sp.	5 ♂ 5 ♀	January

Thus *M. planiceps* is a constant, though numerically not numerous, breeder in fresh cow-dung, which is, in the locality studied, pre-eminently the pabulum of *M. conducens*, forming nearly 92 per cent of all emergences. *M. planiceps*, with second place, forms 82 per cent of the remaining species. The genus *Musca* has—in the Indian Sub-Region,—advanced into viviparity once in the haematophagous and once in the haematophilous group, but not with the most successful biter, *crassirostris*, whilst *bezzii*, in the haematophiles, is a species of rather restricted distribution. The larva of *bezzii* is born in stage II, but that of *planiceps* is born in stage III, in this respect resembling the species of *Glossina*. But whereas the larvae of *Glossina* are full fed and ready to pupate when born, those of *planiceps* are in early stage III and change their pabulum after birth from the maternal secretion to cow-dung and complete their development in this material. This must involve profound changes in metabolism, completely unstudied.

Larval development. The newly emerged female contains only immature eggs. The mature egg passes into the common oviduct, enlarged to form a uterus. A mature egg,

chorion unruptured, has been found in a specimen with fresh blood. A female released 10 hours from eclosion, and unfertilized, has been recaptured 24 hours later with a blood meal already partly digested, and with a larva fairly advanced in development, (but still rather soft and diffluent) and a mature egg. A specimen with a stage III larva was found with an immature egg with no signs of embryonic development and only one more developing egg in the follicles. It would thus appear that as soon as a larva is born the uterus receives an egg ready to hatch from one ovary whilst the other ovary completes the development of the next egg. The female genitalia are figured fig. 227, in Patton and Evans [1929].

The length of time spent by the larva in utero is unknown. The larva lies head forward in the uterus, but turns prior to birth. Mature eggs removed from the mother and placed on saline damped wool do not hatch.

Birth. On arrival the mother fly usually runs about on the surface of the cow-dung mass for about half a minute, examining it. She then chooses for preference a depression in the upper surface of the dung, otherwise the plane upper surface is chosen, but the steep sides of the slope of the pat are usually avoided. Becoming motionless, the tip of the abdomen is slightly depressed. After a short but distinct pause, birth rapidly takes place. The 'after birth', consisting of the chorion of the egg and the moults of the first two instars, intermingled, is attached to the posterior side of the larva at about two-thirds of its length from the head and is rubbed off as the larva enters the dung, remaining on the surface as a white speck. After birth the mother fly is not usually easily disturbed for a few moments, probably due to exhaustion, and may then fly straight off, or stop and feed at the dung.

In nature delivery is almost always head first, exceptions being usually under-sized females, in which there is possibly no room for the larva to turn. But when killed artificially, a fair number of flies give birth tail-end first. In neither case is the well-being of the larva affected: if it occurs in nature the larva turns straight over and burrows, if aborted by maternal death the larva can equally burrow immediately. Larvae can live within a dead mother,—if she is removed shortly after death from the killing bottle—for up to 15 hours. A fly found dead in a cage 14 hours after imprisonment was found on abdominal pressure to contain an unturmed larva, which on expression burrowed immediately. Another fly, killed

in the cage by a hunting spider was found next morning to contain an active mature larva.

The anterior spiracles of the larva appear not to be functional. A larva, half extruded, can stand exposure to cyanide vapour for some-time, but if the posterior spiracles are so exposed for a moment, the burrowing powers are affected.

Larval post partum development. The larva, when born, is white, but when full grown, after clearing the intestine prior to pupation, is clear primrose yellow and rather elongate for a *Musca*. The dung feeding period is normally about two days, but on one occasion it was prolonged to eight days. Pupation occurs in earth beneath the dung.

Puparium. The freshly formed puparium is white, passing within 24 hours through red to dark brown, with pale segmental rings very distinct, making it at once distinguishable from those of other *Musca* species. The pupal period is from 7-17 days, average 9-10.

Emergence. This usually takes place before noon. In flies bred from dung which has been exposed for 24 hours, emergence usually covers three days, on the first of which only a few females appear, on the second the main eclosion of both sexes, and on the third day males only. Often the small purely female emergence of the first day is not seen, and the first eclosions reveal both sexes. Almost always, however, there are some male emergences 24 hours after the last female. At emergence the malpighian tubules are deep yellow,—as is normal in the male—and not pale as in later life. Males over 100 hours from eclosion, and recaptured 96 hours after release, still have the testes paler and more translucent than in captured wild specimens of unknown age.

Imaginal habits. Males have been found, always in the morning, on flowers of *Ocimum sanctum*. Only once has a resting male been taken containing fresh blood. Again only once has a male been seen on a bull, moving about very restlessly, and was not seen to bite.

This last was the only occasion on which a specimen of either sex has been seen on cattle. Placed under netting on a calf, females have refused to feed. But 56 per cent of all captured females taken over six months contained blood in the gut. Against the species being a night-biter is that a few dissections done on flies captured in the early morning have shown the blood always in an advanced stage of digestion.

A specimen of blood submitted to the Imperial Serologist proved to be bovine.

Attempts to feed flies in captivity on human blood exuding from a prick, on raw meat exuding serum, on sugar solution, and on fresh cowdung have all failed, though as noted under larviposition, this is sometimes sucked at in nature.

The species resembles the non-biting haematophiles in that it is never seen so engorged that it cannot fly well, unlike *Stomoxys*, which engorges until it can hardly move, and ingested blood never shows so clearly through the abdominal wall as, for example, in *M. ventrosa*. Even full term females are fairly nimble.

The species always rests on the upper side of grass blades etc.—the only exception to this was a male taken when the weather was just

clearing after a heavy all-day down-pour.

Whatever the species does during the day, and it is very hard to find them, about an hour before dusk both sexes commence to settle on grass in the vicinity of cattle, but whereas breeding results show that the sexes emerge in approximately equal numbers, catching in such a situation produces females to the extent of $\frac{2}{3}$ th of the total. Three-quarters of the males produced are not present, though these observations were made in a spot separated from the next cattle shed by half a mile of a thick stand of rubber trees.

Fortunately adult catches between 5 p.m. and 6 p.m. over the same period as the breeding observations gave the following results:

Caught			Males		Females full term			Females pregnant			Females uterus empty		
♂	♀	Total	blood +	blood —	blood +	blood —	Total	blood +	blood —	Total	blood +	blood —	Total
24	169	193	0	24	50	34	84	32	24	56	13	16	29
Per cent blood +			0	..	59	57	45

The females therefore feed at all stages of gestation. Owing to failure to observe actual feeding, how many meals are necessary to mature a single egg to the third stage larva is not known.

Parasites. *M. planiceps* is parasitized by the Staphylinid, *Aleochara trivialis* Kratz. The method of attack is unknown. The larvae emerge from puparia simultaneously or a day or two later than the flies of the same generation. The bright yellow beetle larvae immediately pupate in earth in a cocoon of soil particles webbed with silk. I have found earth containing puparia felted with their webbing. The pupal period is approximately 16 days.

The percentage of parasitization may be very heavy, varying from 10 per cent in June to 78

per cent in November. The parasite is not always present.

A marked male, recaptured 96 hours after release in October, showed, on the leg lopped through for identification, the fungus *Stigmatomyces baeri*, common on *Musca* species all round the World. The specimen was practically mature, though none of the ascospores had been ejected. Mr. Petch, then Ceylon Government Mycologist, who identified the fungus, informed me that he believed that this observation gives the first record of the duration of development of this fungus.

No intestinal flagellates were found in a pair with empty guts.

REFERENCE

Patton and Evans, (1929) "Insects, Ticks and Venomous Animals", Part I

CHEMOTHERAPY OF HELMINTHIC INFECTIONS OF DOMESTIC ANIMALS

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THE object of this communication is to provide information concerning drugs that have been successfully used in the treatment of ailments caused by helminth parasites in common domesticated animals. References have been made to such drugs as have come into prominence during the past decade and a half. The names of proprietary preparations, whose composition has not been disclosed, have been omitted. References to drugs, which have

been tried *in vitro* only, have been avoided, for when tried *in vivo*, they undergo dilution below the therapeutic threshold, a change occurs in their chemical composition, and the manner of excretion alters their efficiency.

DISTOMIASIS OF RUMINANTS

Hall [1921] used carbon tetrachloride as an anthelmintic with excellent results. Recently it has been proved very emaciacous and sufficiently cheap for adoption in mass treatment, the cost working out at about half-an-anna per dose. The dose for sheep is 1 c.c. and for larger ruminants 5 to 10 c.c. It is given in liquid paraffin in the proportion of 1:4. Other cheap oils, such as groundnut oil or cotton seed oil, may be substituted for paraffin. As only the adult worms in the liver are destroyed by this drug, the dose should be repeated about a month later. It is usually administered orally, but Pegreffi [1939] recommends intra-ruminal injection. He states that pregnancy is not a contra-indication to its use in oily solution. Obitz and Wadowski [1939] treated 3,000 fluke infested sheep by subcutaneous injection of carbon tetrachloride in liquid paraffin. The average time of treatment was one minute per head and the cost was one dollar per 100 sheep. 1,600 sheep were cured with only five accidents.

The drug is an excellent remedy for fascioliasis of ovines, but it is somewhat toxic to bovines. In our experience, however, the bovines recover from the toxic symptoms in about a couple of days. Its toxicity is of a complex nature and mainly consists of the retention in the blood of bile pigments which combine with the ionised blood-calcium and are excreted as such, causing hypocalcemia. It also causes the retention of guanidine in the blood; this interferes with the conversion of glycogen into blood-sugar and causes hypoglycemia. The pathological effect is chiefly on the liver, resulting in central necrosis and fatty degeneration.

Guanidine and calcium are antagonistic to each other in their behaviour and the administration of calcium controls its toxicity; 75 to 150 c.c. of calcium gluconate is administered by injection, or calcium chloride with ammonium chloride or calcium lactate given orally. Injections of sodium xanthine (liver extract) is said to protect animals against carbon tetrachloride poisoning.

Before and after the treatment, the animal should be fed on a carbohydrate diet. Fats and proteins should be strictly withheld, as fats increase the solubility of the drug and cause

its absorption through the lymphatics, while proteins cause an increase in the production of guanidine. As there is a heavy drain on calcium in animals during lactation, greater care has to be exercised in such cases. To decrease the toxicity and to promote a rapid excretion, a dose of magnesium sulphate may be given.

Dicachlorethane (Igitol, Distol) is as effective as carbon tetrachloride and is much safer, but it is somewhat costly. The dose for sheep is 6-8 gm. The doses recommended for the treatment of cattle are 20 gm. per 50 kil. of body-weight, to be given for four successive days in the morning, three to four hours before feeding. The milk of cows undergoing treatment may become slightly tainted, and milch cows fed on concentrates may develop symptoms of colic.

Sprehn (1936) recommends two intra-muscular injections of 20 c.c. of Fuadin for *Dicrocoelium dendriticum* of sheep. On the 10th to 12th day the faeces is completely free from eggs.

GASTRO-INTESTINAL HELMINTHS IN RUMINANTS

These chiefly consist of *Hemonchus contortus*, *Mecistocirrus digitatus*, *Ostertagia* spp., *Bunostomum* spp., *Trichostrongylus* spp., *Chabertia* sp., *Trichuris* sp., *Oesophagostomum* spp., *Cooperia* spp. and tapeworms.

Carbon tetrachloride is very effective against *Hemonchus*, *Bunostomum*, etc., but not against *Trichostrongylus*. This drug can profitably be employed for the combined treatment for fascioliasis and parasitic gastritis.

Carbon tetrachloride in combination with copper sulphate has been used with very good results, and regular monthly treatment produces a marked improvement in the quality of the wool of sheep [Ross and Graham, 1933].

A drug that has come into great prominence during the past four years is Phenothiazine (Thiodiphenylamine— $C_6H_5NHC_6H_4S$). This drug has been known since 1885, but Harwood and collaborators [1938] were the first to use it against nodular worms and ascarids of swine. During the last four years it has been used very extensively in the treatment of gastrointestinal helminths. It has been remarked that the employment of this drug for the treatment of helminths of domestic animals is one of the seven outstanding advances in veterinary medicine. This is borne out by the publication by Professor Leiper [1942] of a special bibliography of 120 references regarding the use of this drug as an anthelmintic. The drug is

administered with food. The dose for sheep is 25-30 gm. and that for cattle 30-50 gm. In some cases the dose may be repeated after a fortnight. It has been claimed that it is 100 per cent effective against *Hemonchus* and *Ostertagia*, 90 per cent against *Bunostomum*, 84 per cent against *Oesophagostomum*, 76-80 per cent against *Trichostrongylus* and only 15 per cent against *Cooperia*.

The anthelmintic action of Phenothiazine is due to thiol secreted into the intestine by way of the biliary tract. Bile facilitates its action. It first becomes leucothiol and then thiol. It is suggested that, since all individuals may not be equally capable of converting it into thiol, the administration of the drug with or without bile will be the next step in the line of treatment.

Apart from its therapeutic value, it also acts as a prophylactic. Phenothiazine-salt mixture offered to sheep for daily consumption in the proportion of 1:9 to 1:14 is effective in preventing the development of parasitic larvae in the faeces. Their development is arrested 48 hours after the administration of 0.5 gm. or more. It thus prevents pasture contamination.

In therapeutic doses it is not toxic, but in doses of 70 to 200 gm. it may cause inflammation of the alimentary canal, tympanites, loss of appetite, weakness of the muscles, sweating, a friable yellowish liver, enlarged kidneys, incoordination of the hind quarters, severe diarrhoea, fever, shock, discharge from the eyes, and death.

A mixture of nicotine sulphate and copper sulphate has proved to be more efficacious than copper sulphate alone, as the combination is effective not only against large stomach worms but also against *Trichostrongylus* and tape-worms. Against the latter it is specific. The mixture is prepared by mixing 1 oz. of 40 per cent nicotine sulphate, 1 oz. of copper sulphate and 3 pints of water. The dose of the mixture is $\frac{1}{2}$ —2 oz. for sheep and for cattle 1 oz. for each 50 lb. of body-weight, with a maximum dose of 3 oz. In this combination the safety factor is lower than in copper sulphate alone, and for this reason the dose recommended should not be exceeded.

Carlson [1939] recommends stabilizing the above mixture, at a dilution twelve times stronger than the therapeutic dose, in gum arabic. This greatly facilitates its transfer and storage for the treatment of large numbers of animals.

Experiments in the Punjab indicate that a mixture of copper sulphate and kamala is the best treatment for haemonchosis.

The use of unstandardized tobacco infusion, common in this country, is to be deprecated. Until the nicotine contents of crude tobacco is ascertained, the dose may prove either excessive or insufficient. This fact requires emphasis, as nicotine is very toxic to animals.

Tetrachlorethylene is similar in action to carbon tetrachloride and, although not so effective as the latter drug, its safety factor is relatively high. The dose is up to 10 c.c. Three such doses should be given at 10 to 14 days' interval. A mixture of carbon tetrachloride and tetrachlorethylene has been claimed by some workers to be safer and more efficacious, but Rose and Gordon [1935] do not find it to be of special value. The toxicity of tetrachlorethylene can be overcome by using a soap emulsion of the drug.

Mönning [1935] has recently developed for oesophagostomiasis an excellent remedy which has been used extensively in South Africa. It consists of copper arsenate one part, copper tartrate one part and calcium hydroxide two parts by weight. The dose ranges from 0.25 to 2.5 gm., depending upon age. The animals are dosed on a full stomach. Repetition of the dose, once in three weeks, has proved to be effective for *Oesophagostomus* and *Hemonchus*.

Lead arsenite in doses of 0.5 gm. to lambs against *Moniezia* is both effective and cheap.

Gordon [1935] has found arsenic trisulphide and arsenious sulphide 100 per cent effective against *Moniezia* in sheep. The dose is 0.5 gm.

The addition of 2.5 to 3 kilograms of anhydrous sulphuric acid to 1,000 litres of drinking water is claimed to be both curative and prophylactic for parasitic gastritis.

Three drachms of deodor oil with 2 dr. of *spiritus atheris vitiosi* in 4-6 oz. of linseed oil is said to be effective against ascariasis of buffalo calves.

Swales [1941] recommends the following seasons for treatment of sheep in Canada: for haemonchosis, summer; for trichostrongylosis, late autumn; and for oesophagostomiasis, autumn and winter.

The anthelmintic treatment of ruminants has been much simplified in recent years in that the troublesome process of previous starvation has been discouraged, since it has been found that withholding food, even for as long as 48 hours, does not empty the rumen; food still continues to pass into the abomasum, so that the efficiency of the drug is not at all altered.

Another great advance made is the discovery by Ross [1936] and by Mönnig [1935] of the

value of giving 2.5 c.c. of a 10 per cent solution of copper sulphate to sheep immediately before the administration of a drug. This stimulates the vagus of the pharynx and brings about the closure of the œsophageal groove. It has thus become possible to administer drugs directly into the abomasum which they reach in full concentration, deriving the maximum benefit. It must be remembered, however, that the reflex lasts for only 15 seconds, so that the anthelmintic should be given immediately after the administration of the copper sulphate solution.

VERMINOUS BRONCHITIS OF RUMINANTS

Camus and Chevalier [1931] state that pyrethrin dissolved in oil is a very efficient remedy for verminous bronchitis of cattle. Ten c.c. of an aqueous solution of 0.01 per cent pyrethrin given intra-tracheally is effective against *Dietyocaulus filaria*. The drug can be administered through the nose by a rubber nozzle measuring 12 to 15 cm. the sheep being kept in a sitting position and thereafter put on its back for a few seconds. This procedure may be repeated twice, if necessary.

Two intra-tracheal injections of 5-10 c.c. of triformal-glycerine-allylique was found to be more effective against *D. filaria* than any well-known drug. It was observed that the use of isotonic solution at about blood temperature obviated the reflex cough which usually follows the injection.

Picric acid is recommended for controlling the lung worms of sheep and goats. It is neutralized by N/10 potassium hydroxide solution. Five injections of 10 c.c. of a 2 per cent solution on alternate days is effective for verminous bronchitis of buffaloes.

Injection of 10 c.c. of a 10 per cent solution of chloroform is recommended for *D. viviparus* of cattle. It may, however, prove dangerous.

Fumigation with chloropicrin has also been recommended for bronchial worms.

HELMINTHIASIS IN HORSES

Phenothiazine is stated to be 100 per cent effective against *Strongylus*, *Trichostrongylus*, *Trichonema* and *Oxyuris* spp., and 72 per cent against *Ascaris equorum*. The dose for heavy draughts is 2 oz., for light to medium 1.5 oz., for thorough-breds 1 oz. and for small horses 0.5 to 1 oz. It may be administered with liquid by a stomach-tube or fed with a large quantity of bran. In very heavy doses it is toxic, and the main features of the toxicity caused are the loss of equipose, nervous dis-

orders, dermatitis, necrotic enteritis, albuminuria, hæmoglobinuria, nephritis, abdominal pain, bronzed mucous membranes and painful micturition.

Carbon tetrachloride in doses of 10 c.c. per 100 kil. of body-weight (50 per 1,000 lb.) is very effective against blood-sucking strongyles. It is administered by a stomach-tube. Care should be taken that the tube is well inside the stomach, lest the drug should be regurgitated and inhaled with fatal results.

Dichlorobutane or chlorobutylene in doses of 0.15 to 0.2 c.c. is stated to be very valuable against cylicostomes.

Oil of chenopodium is stated to be particularly effective against red worms. It is administered either by a stomach-tube or in a drench mixed with linseed oil. Full doses should not be given to highly-bred horses, and like carbon tetrachloride, it is contra-indicated in febrile conditions, gastro-enteritis and in cases of constipation.

Normal-butyl chloride has been found to be very effective against *Strongylus* spp. and *Oxyuris equi*. The dose is 0.1 c.c. per lb. of body-weight. The administration of the drug should be preceded by a fast of 36 hours and followed by a drench of eight to ten times its volume of linseed oil.

Carbon disulphide is specific against habronemiasis and ascariasis. The animals are starved overnight, and in the morning 10 litres of a 2 per cent solution of sodium bicarbonate is passed through a stomach-tube in order to loosen the worms from the mucous membrane. Thereafter as much of the fluid as possible is removed, and 5 c.c. of the drug per 100 kil. of body-weight is administered. The worms inside the tumours are difficult to remove.

Boley and collaborators [1941] recommend that a mixture of 40 gm. of phenothiazine and 24 c.c. of carbon disulphide, administered in a capsule, after 36 hours' fast, is very efficient against ascarids, strongyles and bots.

Petrol was used as an anthelmintic some fifty years ago by Boley [quoted by Faure, 1940]. Faure [1940] tested it in horses and found it very effective against *Ascaris* and *Oxyuris*. After fasting, a dose of 0.5-0.7 c.c. per kil. of body-weight should be given in three daily doses, mixed with culinary or castor oil and followed by water. The drug is easily tolerated and is free from contra-indications. Faure cites a case in which 1,100 ascarids were evacuated in one night.

Nörr [1940] reports that santostibin is very effective against *Ascaris equorum*. Two to

three doses of 15 gm. each or three to five doses of 10 gm. each should be given in bran.

Intravenous injections of Stavarsol destroy the larvae of strongyles and cyclostomes. The dose is 5 gm. repeated three to ten times.

Lang [1936] claims that, intravenous injections of 5 per cent sodium chloride and 5 per cent sodium citrate, in 2 pint doses, destroy the thrombus formation.

HELMINTHIASIS IN DOGS

Hexyl-resorcinol has been shown to be effective against ascarids and hookworms. It is irritating to the mucous membrane and should be administered as a powder. It decomposes gelatin and is ineffective when mixed with oil. It is administered in freshly filled gelatin capsules or in sugar coated tablets. The dose is 250 mg. per kilo. of body-weight.

Hexylmetacresol is said to have all the good, and none of the bad, qualities of hexylresorcinol.

Carbon tetrachloride has been found to be very effective against hookworms and has in recent years become very popular. The dose is 0.3 c.c. per kilo. of body-weight and is administered in gelatin capsules, with the necessary precautions (see distomiasis).

Some workers claim that a mixture of carbon tetrachloride and oil of chenopodium in the proportion of 2 c.c. of carbon tetrachloride and 20 min. of oil of chenopodium is more effective than the former alone. Both drugs can be given mixed in the same capsule, followed an hour later by a dose of magnesium sulphate. It is said that the drugs are supplementary to one another, and for this reason the dose of each individual drug can be reduced. The toxicity of the combination is not greater than that of the drugs themselves when administered separately.

One c.c. of chloroform mixed with 30 c.c. of castor oil has been recommended for ancylostomiasis.

Phenothiazine is useful for ascarids, hookworms and whipworms. The dose is 3-10 gm. It is not toxic.

The injection of at least one gm. of phenothiazine in the vicinity of a *Dracunculus* ulcer reduces invalidism and hospitalization. The drug should be made into an emulsion with olive oil, water and *adepts lanx*.

Prontosil, in combination with emetine hydrochloride, brings about a rapid and radical cure in dogs infested with *Paragonimus westermani*.

Hydrogen peroxide, sodium permanganate and sodium hyperborate are said to be effective

against most of the worms of dogs and are quite safe. It has been claimed that a rectal injection of 1.5 c.c. removed all tapeworms (either vomited or passed in faeces) and most other worms. There were no ill effects.

Arecoline hydrobromide has proved to be the drug of choice in the treatment of teniasis of dogs. The dose is 2 mg. per kilo. of body-weight. The drug frequently causes nausea and vomiting and is best administered in gelatin capsules together with chlorotone (about 5 grains per 10 kg.). The worms are passed within 10 to 20 minutes. Nosik [1940] states that arecoline hydrobromide in doses of 0.002 to 0.003 gm. per kilo. followed by the extract of male fern in a dose of 0.2 to 0.3 gm. per kilo. and repeated after 10 days is 100 per cent effective against *Echinococcus granulosus* and other *Tenia* spp. It has some effect against ascarids, but is ineffective against *Mesocostoides*.

Iso-amyl-ortho-cresol is specific against *Dipylidium caninum*. The dose is 0.1 c.c. per lb. of body weight, followed by a dose of magnesium sulphate.

Brooks and Brown [1942] consider that ficin is 100 per cent effective against ascarids, 96 per cent against *Trichuris dispar* and only 13.6 against *Ancylostoma caninum*. It is totally ineffective against *Dipylidium caninum*. The dose is 750 to 1,000 mg. of the drug per kilo. of body-weight to be given as a 15 per cent aqueous suspension by a stomach tube, followed, three to four hours later, by 1 gm. of Epsom salt per kilo. in a saturated aqueous solution.

Gentian violet has been recommended for *Strongyloides stercoralis* of dogs as it has been for man. The dose is 15 to 17 mg. per kilo. body-weight.

N-butyl chloride in a dose of 0.3 c.c. per kilo. body-weight is useful against hookworms and ascarids. Wright *et al.* [1933] states that a rectal injection of 1 oz. per 20 lb. of body-weight removes all caecal worms.

N-butyl bromide and N. amyl bromide are stated to be 100 per cent effective against *Ancylostoma caninum*.

'Rotenal' (the active principle of *Deguelia*) is useful against hookworms and ascarids of dogs, in doses of 0.2 gm. per kilo. of body-weight.

'Nemural', a pyridine derivative, is effective against tapeworms of dogs. The dose is 18 mg. per 15 lb. of body-weight. A tablet dissolved in water can be given after a light meal and should be followed by an enema. No laxative is necessary. The drug is not toxic. The tapeworms, especially *Dipylidium caninum*,

are removed within 5 hours.

Pumpkin seeds are useful against tapeworms of dogs and man. The dose for dogs is 5 gm. and for man 30 gm., and its administration should be followed by a purgative.

The following drugs have proved to be effective against the heart worm of dogs, *Dirofilaria immitis*:

(1) According to Brown and Sheldon [1941], a ten-day course of daily intra-muscular injections of 1 to 2 c.c. of Fuadin and the oral administration, twice daily, of 20 to 25 gm. of sulphanilamide. The onset of otitis indicates the death of the parasites.

(2) Five injections of 6 c.c. of Antimosan at an interval of three to four days. [Kaka, 1937].

(3) 'Stibsol' (antimonian-3-catechol-thiosalicylic acid-sodium, containing 30 per cent of antimony) has been recommended by Brown and Austin (1939). It kills the female worms in the right side of the heart of the dog, and the dead worms are absorbed by the lungs. It also removes microfilariae from the circulatory system. Two or three intra-venous injections are sufficient. The treatment is equally effective against heavy and light infections.

HELMINTHS OF POULTRY

Carbon tetrachloride is very effective against the large round worms of poultry. Tetrachlorethylene has also been claimed to be a good remedy, but it is not so effective as the former drug. The dose is 1 to 2 c.c. with 3 to 4 c.c. of oil. Both are powerful agents, and therefore over-dosing should be avoided. The drug should be administered in a gelatin capsule or through a flexible rubber tube introduced directly into the crop.

Colloidal iodine preparations are becoming popular and have proved satisfactory. A 10 per cent solution in water injected into the gizzard, in doses of 1 oz. to a bird weighing 4 lb., is stated to have removed 89 per cent of nematode, and cestodes. The worms evacuated were completely dead. Stafseth and Thompson [1932] claim it to be 100 per cent. effective against tape and round-worms.

Phenothiazine in doses of 0.2 to 0.5 gm. is satisfactory for *Heterakis* and *Capillaria*.

Bleeker and Smith [1933] claim that a mixture of nicotine sulphate and kamala, in the proportion of one-fourth drachm to 0.2 c.c., is 100 per cent effective for all common worms of fowls, with the exception of caecal worms.

Levine [1938] states that the areca nut is the

only successful drug for the removal of *Davainea proglottina*. It removes all scolices. The dose is 2 gm. to a chicken.

Synthetic pelltierine, tin oleate, stannous oxalate and tetra-iso-butyl-tin remove *Raillietina cesticellus* and *Hymenolepis carioeca* of chickens.

Recently Harwood and Guthrie [1940] have struck a despondent note regarding all treatments of poultry teniasis. They tried 223 substances and 27 mixtures. Of these only lead arsenate exhibited any promise as a poultry teniacide, but it was found to be too toxic in effective doses to be recommended for general use.

Pyrethrum powder containing 8 per cent pyrethrine is considered to be 95 per cent effective against *Ascaridia galli*. The dose is 200 mg. to be administered in gelatin capsules. An aqueous solution of the drug, 1 in 100, is also used administered through a gum elastic tube.

N-butyl chloride is stated to be very good for *Ascaridia*. The dose, according to Wright and collaborators [1935] is 1-6 c.c.

The following drugs have been stated to be very effective against gapeworms:

Clapham [1935] recommends the administration of garlic oil or the synthetic product, allyl sulphide, into the trachea by means of a pipette. The dose ranges from $\frac{1}{2}$ to 3 min. to be given as a 33-3 to 50 per cent solution in linseed oil. The drug is very powerful, ruptures the worms and renders the eggs sterile. Allyl sulphide is cheap and less pungent.

Delaphane and Stuart [1939] treated gapeworms of pheasants with 100 per cent success by instilling a few drops of iodine vermicide into the trachea.

Wehr and collaborators [1939] found that the dusting of birds with barium antimony tartrate powder is more than 98 per cent successful against gapeworms. The drug is automatically inhaled.

Our experience at Mukteswar shows that birds fed on bits of raw garlic carry very little worm burden.

CUTANEOUS HELMINTHIASIS

For parafilariasis, Losev and collaborators [1937] recommend subcutaneous or intravenous injections of an aqueous solution of iodine and the washing of the affected areas with 1 per cent phenol. Intravenous injections of 100 c.c. of 2 per cent aqueous extract of tartar emetic have also been recommended.

The drugs recommended for stephanofilariasis are Cooper's 'Laveno' or Cooper's 'Dip' and intravenous injections of tartar emetic.

Against habronemiasis, intravenous injections of Antimosan or tartar emetic are given. The following powder is applied until the wound is healed: Plaster of Paris 100 parts, naphthalene 10 parts, alum 20 parts and quinine or bitter powder 10 parts.

NASAL SCHISTOSOMIASIS

Intravenous injections of 1.5 grains of antimony tartrate for every 100 lb. of body-weight of the animal are repeated daily for six days or 2.5 grains every alternate day.

GENERAL

One notable discovery that has been made during recent years is the addition of charcoal to santonin, oil of chenopodium or carbon tetrachloride reduces the toxicity of these drugs, without interfering with their anthelmintic efficacy.

An automatic drenching gun has been introduced for administering fluids to sheep. The apparatus consists of a gun with knapsack container. It is adapted to give doses of 1-2 oz.

Mention may be made of the methods adopted by Moskey and Harwood [1941] to evaluate the efficacy of anthelmintics. The tests may be grouped under *in vitro* and *in vivo* tests. The latter may be sub-divided into (1) dilution egg-count test, (2) the critical test and (3) the controlled test.

In vitro tests: they cannot be accepted until adequate *in vivo* tests have been performed. For example, pineapple juice dissolves ascarids *in vitro* but the juice has no effect on ascarids *in vivo*.

In vivo tests: the drug which is useful for one kind of worms in one animal may not have the same efficacy in other hosts.

Dilution egg-count test. Stoll's method cannot be used as a true indication of the anthelmintic efficacy of a drug, since the drug may reduce the reproductive capacity of the worms without eliminating them. This method cannot be used in testing the effectiveness of a tennicide or of indication aimed at parasites whose eggs do not appear regularly in the faeces.

Critical test. This was first advocated by Hall. It consists in counting the number of worms in the total amount of faeces passed on three or more days and then adding to the result the total number of worms obtained at

necropsy. This test must be repeated on a sufficiently large numbers of animals. Flaws: (1) Worms may be eliminated by other cause than anthelmintics, (2) the killed worms may be digested by their hosts, and (3) these tests are not useful in the case of tennicides. The scolex may not be eliminated. The animals should therefore be retained for two or three weeks to allow the worms to grow. Occasionally, the scolex may be destroyed, presumably by digestion after it is killed.

Controlled tests. These are done in cases of artificial infection. In the case of fowls, 10 birds should be used for artificial infection and 10 as controls.

Safety. No drug should be advocated unless its therapeutic index is known.

Finally mention should be made what Hagen [1936] considers to be the ideal anthelmintic. It contains paracymene, thymol, castor oil and kamala. It has been claimed that it has efficacy against all species of worms and is of low toxicity.

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DETERIORATION OF CATTLE IN CERTAIN PARTS OF INDIA AND ITS PROBABLE CAUSES WITH SOME PRACTICAL SUGGESTIONS TO OVERCOME THEM

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(With three text-figures)

THE existence of good and bad areas for cattle in India can easily be mapped out and correlated with certain types of cultivation. Good breeds of cattle are confined to comparatively dry areas such as Sind, the Hariana and Dhanni tracts of the Punjab, Rajputana States, Kathiawar and in such parts of other Provinces where similar conditions exist. Pasture in these dry areas may be good in quality but is often scarce and the uncertainty of rainfall makes it obligatory on the part of zamindars to grow crops, the residue of

which provides a good supply of fodder for cattle. Conversely in tracts with a humid climate which are subject to heavy rainfall or are provided with ample irrigation water a very poor type of cattle is found, in spite of the availability of grazing. In these areas, cash crops are given the most attention and fodders suitable for cattle are seldom grown (Figs. 1 and 2). Other areas which fall midway between these two possess cattle of intermediate quality and the reasons for deterioration are not so easily explained.

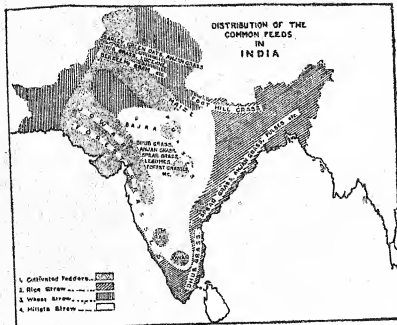


FIG. 1. Distribution of common cattle feeds in India

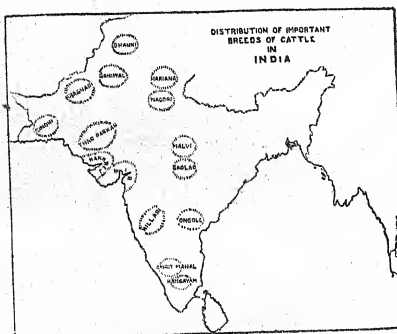


FIG. 2. Distribution of important breeds of cattle in India

It is a well-known fact that in the rice growing tracts and flooded areas of Assam, Bengal, Bihar, Orissa, Madras, Travancore, etc. cattle are generally found to be greatly deteriorated, both as regards their physical development and capacity for work and milk production. They are also more prone to diseases, and reproductive defects are common in them. It is also a common experience in these tracts that when foreign animals of any breed are introduced for the grading up of local cattle they deteriorate rapidly. In other scattered rice growing areas like Kangra district in the Punjab, Gorakhpur district in the United Provinces, Malabar coast of Madras, Chhattisgarh district in the Central Provinces, a similar type of deteriorated cattle is found. In these areas the cattle being inefficient, the number of cattle

required for agricultural purposes and for milk production is necessarily larger, even three to four times as compared to that of other areas where good cattle exist. The larger number of cattle causes greater strain on the available pastures which results in their further deterioration, thus producing a vicious circle.

In the States of Travancore and Cochin where the cattle are of a mongrel, non-descript type and where there are no recognized breeds, cows are notoriously poor milkers, the yield obtained being not more than that of a good goat elsewhere. The bullocks are puny and weak and on this account the ryot is generally unable to adopt and make use of modern, efficient and labour-saving implements. To improve the agricultural practices and provide more milk for the people the Travancore Agricultural Department imported bulls from the adjoining British Indian districts and Indian States, with a view to grade up local cattle. The Ongole, the Kangayam and the Amritmahal were the chief among these breeds. These carefully selected bulls were stationed at the Government Cattle Farms to serve the farm cows as well as the cows belonging to the local public, but the bulls did not thrive well and soon deteriorated. The calves born to them were also not satisfactory. They were, therefore, discarded. Yet another attempt was made and bulls from upper India belonging to the Sahiwal, the Sindhi and English cross-breeds were imported and tested. Of all these breeds, the Sindhi, a medium sized breed, has fared the best and appears able to withstand the local conditions admirably.

The United Provinces has been divided into five definite tracts according to the types of cattle found :

(i) The dry western tract (rainfall 20-30 inches) which includes the area west of the Ganges, stretching from Cawnpore to Saharanpur, including 14 districts which form part of the Agra, Meerut and Allahabad Divisions. In this tract good types of cattle, sheep and goats are to be found, and the soil and climatic conditions of the tract lend themselves to improvement being effected in the existing types.

(ii) The central humid tract (rainfall 30-45 inches) consisting of the highly cultivated areas of parts of the Rohilkhand, Lucknow and Fyzabad Divisions. In this tract only animals of moderate size and mediocre efficiency in draft and milk are produced and better classes of animals, if introduced, tend to degenerate.

(iii) The *tarai* tract, situated at the foot of the Himalayas (rainfall 45-65 inches). This tract includes areas where grazing is in abundance, but only small types of draft cattle can be raised. This tract has no cattle or buffaloes of milk type and better types of cattle degenerate rapidly.

(iv) The Bundelkhand area of many different soil formations where types vary noticeably with the different types of soils.

(v) Hill tracts which can produce only the poorest and smallest types of cattle and where cattle from other tracts tend to rapid degeneration.

Within the above tracts, however, there are to be found some areas which, though situated in a tract where generally poor types of cattle are raised are favoured in some way or other by suitable soil and fodder conditions and are, therefore, able to produce within these circumscribed areas cattle much superior in type to the general type of the tract.

In the rice-growing tracts of Bengal and Bihar, the condition of the cattle is very similar to that seen in other rice-growing tracts of India. Poor specimens of small stunted animals are commonly found in these areas. In Bihar, inspite of availability of plenty of grazing in the region where the Kosi river flows, the cattle and buffaloes are of a degenerate type, the average milk yield of cows rarely exceeding one seer a day and that of buffaloes varies from two to five seers a day.

At the Military Dairy Farms in the Punjab, Sind, North-West Frontier Province, and Baluchistan, it is said that the cattle do not appear to suffer from any mineral deficiency. There is some evidence to show that cows in the United Provinces and the Central Provinces benefit by the addition of minerals to their diet. Generally, yields of cows increase as one travels north and east of Delhi upto a height of 5,000 feet but excluding a narrow strip at the foothills of the Himalayas. In addition, the high country in the Central Provinces and the Deccan is fairly good in certain localities, for example Jubbulpore and Secunderabad, although it rains too much at Jubbulpore and cows fall off in condition during monsoon. The United Provinces is not good and gets worse as one travels towards Bengal. Coastal areas are bad, until Karachi is reached which area is fairly good and improving as one travels north. Ruk and Rohri are too humid for cows due to the Indus river. In this broad outline, there are, of course, certain local exceptions. High humidity combined with high temperature has been found to be the worst combination. In the opinion of many, no milch animal can stand the strain of producing large quantities of milk in such a climate for long and the breed deteriorates. The best buffaloes are bred in fairly dry localities and the ideal locality for buffaloes appears to be a place where there is a temperature of about 100°F. in the day and about 70°F. at night, lots of green fodder, coarse if necessary, and opportunity to wallow during the major portion of the day.

The deterioration is not confined to individuals only but also extends to their progeny, thus

causing a long range drop in efficiency of incalculable magnitude. The under-fed bullock is unable to perform work satisfactorily, the semi-starved bull is incapable of producing strong and virile progeny and the ill-nourished cow gives very little milk. Besides the loss in milk yield, the cow under such conditions of malnutrition does not breed properly or regularly, often brings forth weak and sickly or dead calf and thus causes a further deterioration of the stock. All these under-fed animals fall easy victims of the ravages of disease and thus causes further expense and loss. This deterioration of cattle has a far-reaching effect on the agricultural operations and human nutrition and health. In the words of Sir Robert McCarrison [1927] it must be realized that the problems of animal husbandry are also the problems of human husbandry.

In these notorious areas, improvement of cattle cannot be effected by any method of breeding, unless the causes of deterioration are removed. The scientific demonstration of a definite cause or causes for a condition offers the best chance for introducing preventive and curative treatment. The problem of deterioration of cattle is a very vast one and its causes may be many.

From the information available from other countries, it is probable that diseases and internal parasites like helminths, intestinal and blood protozoa, etc. play an important role in livestock deterioration and bring about heavy losses, though there can be no doubt that the low nutritive value of the natural pasture is another main cause of stock deterioration, especially amongst village cattle in India which depend almost entirely on grazing. The endocrines as affected by the deficiency of minerals, vitamins and proteins, trace elements like cobalt, manganese, fluorine, selenium, etc., and environmental factors such as climate, humidity, temperature, rainfall, etc. also significantly affect the health of cattle.

REVIEW OF WORK DONE

A. Foreign countries

A review of available literature on the subject shows that many notoriously bad areas for cattle breeding are known in Africa, Europe, Australia and other countries. A revolution has followed the discovery of phosphorus deficiency as the immediate or ultimate cause of serious losses in this branch of Agriculture. Extensive research work has been carried out on the various aspects of the problem and it will be of interest to review the same briefly in this note.

It has long been known that food must contain lime and phosphorus for the formation of bone and other inorganic salts for the blood and soft tissues. As early as the beginning of last century it was recognized that the lack of these might be

the cause of abnormal conditions in sheep and cattle. By the middle of last century it was proved that some of these conditions in cattle, occurring in certain districts in Europe, and especially in the winter months and in seasons of drought, were due to the lack of either lime or phosphorus in the pasture or fodder.

In parts of South Africa, Australia and the United States lack of phosphorus in the pasture is associated with a disease which goes by various names, but is characterized during the life of the animal by stiffness of gait, slow growth, poor breeding capacity, general unthriftiness and *pica* (i.e., a 'depraved' appetite for substances such as old bones or earth) and on *post-mortem* examination, by a soft or brittle condition of the bones.

It has been shown, under experimental conditions, that lack of lime in the rations of cows may be the cause of failure to breed successfully, the calves being either born dead or with such low vitality that the death rate is high. Lack of sodium and chlorine has been proved to be a cause of unthriftiness in milk cows. Iron and iodine are also needed by the animal, but only in traces.

It is believed, however, that deficiencies, less marked than those referred to above as the direct cause of certain abnormal conditions, may exist and be the cause of a degree of malnutrition which affects the rate of growth and production, and may possibly render the animals more susceptible to specific diseases. Orr [1930] has suggested that in order to balance up the rations with minerals one should calculate from the known composition of the carcasses of animals or of products such as milk, the amounts of each of the different minerals which must be absorbed from the intestine and retained in the body for the building up of the bones and other tissues in growth, or used for the elaboration of products such as milk. For example, calcium (calculated as CaO) is present in the bodies of cattle to the extent of about 2 per cent and the percentage of phosphorus (calculated as P_2O_5) is only slightly less. Therefore, young cattle putting on 2 lb. per day live P_2O_5 weight would need to absorb and retain more than $\frac{3}{4}$ oz. of lime per day. Every gallon of milk contains about $\frac{1}{4}$ oz. of lime and $\frac{1}{4}$ oz. of phosphoric acid; hence a cow giving four gallons of milk a day would lose each day about 1 oz. of the former and $1\frac{1}{4}$ oz. of the latter in the milk. Only part of what is contained in the food is absorbed from the intestine and even what is absorbed is not all available for formation of new tissue or for milk, because there is a continuous loss through excretion. Hence, the food must contain more than is required to be retained in the body or secreted in the milk. To make up for calcium and phosphorus deficiencies, ground limestone and steriliz-

ed bone meal may be used respectively. Fraser [1935] has stated that wild herbivora and native breeds of cattle seem to be less liable to the effects of mineral deficiency than imported breeds of high fertility, rapid growth and heavy milking type, because such functions require more phosphorus than the natural pastures of a large area of the world can supply and therefore the improved breeds may die, lose type or revert to scrub under less favourable conditions. Progressive depletion of minerals in pasture lands exerts quite an apparent influence on the general well-being of the stock resulting in conditions like osteophagia, delayed maturity, etc. He has suggested that the mineral deficiency can be combated by supplying the deficient mineral element in adequate amount either by direct feeding or indirectly through crop by manuring the soil, the latter appearing theoretically a more natural method.

Recently du Toit, Louw and Malan [1941] have published their final report on a study of the mineral content and feeding value of natural pastures in the Union of South Africa. In this study, originally it was decided to analyse the soil from selected areas, the herbage growing on the soil and the blood of cattle grazing on the pasture. It was thought that a definite correlation existed between these three sets of data. But after the first three surveys which were reported on separately by du Toit *et al* [1932], the collection of soil samples was discontinued. The results warranted the conclusion that soil analysis does not provide a satisfactory method for studying the feeding value of natural pastures, as pasture on poor soil may yield excellent values if the samples for analysis are taken at an early stage of growth of the pasture. 'Animal' samples of natural pasturage were collected and chemically analysed. These samples were collected by following the animals at pasture in order to ensure that such samples were as closely as possible representative of what the animals actually consumed on the day of collection. Judged by the estimated requirements of cattle and sheep for growth all South African natural pastures, composed mainly or wholly of grasses are deficient in phosphorus, crude protein and in certain areas, sodium for a period ranging from five to nine months of the year, depending on the area. There are indications that in certain of those regions the pasture may be deficient in phosphorus also throughout the year. Furthermore, on the basis of the average value for phosphorus, these grass pastures contain at no time of the year sufficient of this nutrient to provide in the requirement for an additional function (e.g., gestation or lactation) of the animal, superimposed on growth. Theiler [1934] described in detail the clinical and histopathological features of the skeletal diseases of animals caused by mineral

deficiency or imbalance and has cleared up the existing confusion in their classification by using the terms in their precise histopathological sense. He has also suggested that the endocrine mechanism may be of significance in controlling the mineral metabolism, a point which needs further research.

Humphrey [1931] has remarked that vitamins are substances of very pronounced physiological activity, and can produce remarkable changes in the normal body, even in minute doses. An adequate supply of vitamin A in the blood is necessary to maintain the mucous membranes in a stage of full physiological activity, including their functions of preventing bacterial infections and parasitic infestations. Vitamin D can be used with advantage to increase the bactericidal power of the blood and is of importance in connection with calcium and phosphorous metabolism. Vitamin E appears to be essential for normal reproduction and lactation. It is found in the unsaponifiable fraction of wheat-germ oil, and shows close relationship to the group containing Vitamins A and D.

Green [1938] has reviewed the importance of trace elements in veterinary science. Many of the obscure diseases have now been attributed to the trace elements. Since plant food is the basis of animal life, any trace elements present in the plant are likely to be found in traces in the animal body as well, where they may exercise a beneficial effect (e.g., copper, cobalt, manganese) or a harmful effect (e.g. fluorine, selenium and molybdenum) or be entirely inert (e.g. boron). 'Nutritional anaemias' described under various names in various countries were considered to be due to iron deficiency and prevention of the disease could be achieved by providing licks of crude iron compounds. But, it is now known that catalytic trace elements are frequently involved in the aetiology of these naturally occurring conditions—sometimes copper, sometimes cobalt and sometimes both apart from ensuring sufficient iron for actual haemoglobin formation. Stewart and Ponsford [1940] have stated that although administration of cobalt has failed to cure pining, the possibility is not ruled out that specific deficiency of this element may play an initial part in some areas in the cause of pining by lowering resistance to parasitic diseases. Manganese is now regarded as an essential trace element for animal life, appears to stimulate growth, is involved in haematopoietic function, in skeletal metabolism and in reproduction. An excess of fluorine in drinking water interferes with bone metabolism and causes dental defects, osteoporosis, etc. An excess of selenium is manifested in cattle, horses and swine by a depressed rate of growth, loss of hair, most conspicuous in the mane

and tail of horses, abnormal development and sloughing off of hoofs. Watson [1938] has suggested that the scouring and reduced milk yield of cows in certain seasons may be caused by a higher content of molybdenum in herbage of certain grazing areas of Somerset. Boron is known to be essential for plant life and hence finds its way in traces into the animal economy where, however, there seems to be as yet no proof that it performs any specific function. Traces of zinc, which seems to function as a stimulant to the growth of certain crops, increasing chlorophyll production and photosynthetic activity etc., would naturally find their way into the animal economy and are apparently sufficient since no naturally occurring zinc deficiency disease" has been reported, although rats experimentally fed upon a purified diet containing less than 0.02 mg. per day showed delayed intestinal absorption and decrease in growth rate.

Cuthbertson [1940], while discussing the quality and quantity of protein in relation to human health and disease, has stated that although the adult physical pattern is determined by hereditary influences, yet in the period of growth it may be enhanced, dwarfed, warped, or mutilated by the influence of the environment of which man's food supply and its protein element form a part. McCay [1912], quoted by Cuthbertson, states that there is ample proof of the all-important influence exerted by food and particularly protein, in determining the degree of muscular development, the general physical endowment, the powers of endurance, resistance to disease, and most of all the place a tribe or race has won for itself in manliness, courage and soldierly instincts, etc. The higher the level of protein interchange, the more robust and energetic, and the more manly the race. The density of population in relation to available food supply determines the protein intake. Low protein in diet reduces an animal's resistance to disease and retards the healing of wounds and prolongs convalescence.

Du Toit *et al.* [1941] have concluded that since, unlike phosphorus or calcium, the reserves of which in the animal's body may tide it over a long period of inadequate intake without appreciably affecting its productive performance, protein undernourishment may immediately limit production or prevent it entirely. The low level of this constituent existing for several months in each year in most natural pasturages must be considered the limiting factor in the productivity of both cattle and sheep in the Union of South Africa. They have further stated that the extreme deficiency of protein in the grass pastures during winter is a problem of equal, if not greater importance than that of phosphorus deficiency and they surmise that the improvement or

supplementation of winter pasture with the object of increasing the protein intake of animals should greatly benefit the cattle and sheep industries of their country.

Leitch [1937] has remarked that in addition to the direct influence of under-nutrition or malnutrition due to imbalance of the constituents of diet, vitamins, proteins or minerals on growth, fertility and production, there is the other, possibly even more interesting and important, indirect effect through its modifying action on susceptibility to infections. There is evidence that it may be an important pre-disposing factor in infection with *Br. abortus*. Ross and Graham [1933] have specifically investigated the effects of the improved pastures in (a) increasing the degree of parasitism owing to the heavier stocking carried, or (b) so improving the nutritional state of the sheep that the animals are able to resist infestation or at least the effects of infestation. Ross and Gordon [1933] pointed out that in sheep kept on a diet low in protein and minerals the resistance to *Haemonchus contortus* was completely broken down.

Richardson [1935] has stated that it may be that climatic factors affect the protein and mineral contents of a pasture and the difference in mineral content of pastures in tropical and temperate regions may be caused by a differential effect of climate on the rate of nitrogen and mineral uptake on the one hand and rate of growth on the other. Bonsma [1940] has discussed the influence of climatological factors on cattle and is of opinion that livestock react strongly to climatic changes, because these changes affect their vital physiological functions such as internal combustion or metabolism, respiration, body temperature, habit, fertility, etc. The activity of endocrines like the pituitary and adrenal glands is also affected. The problem of under-development of cattle in tropical and sub-tropical regions is closely connected with the climate and temperature of the regions in which the animals are kept. Teodoreanu [1938] has reported that domestic animals brought from one region to another show variability of different degrees depending on the influences of climatic and food changes on their constitution, resistance, production, etc. It is possible to distinguish three types of such modifications.

1. In the first, the animals are subjected to non-hereditary modifications which can be observed and appraised by a competent eye and combated by prophylactic measures. The different parts of the body are subjected to slight diminutions from the original size. Robustness suffers as well as the productions of milk and beef.

2. The second class embraces animals showing hereditary variations during several generations. Constitution and production diminish until approaching that of the local breeds.

3. The third class embraces those animals that fail to become acclimatized. These degenerate gradually and disappear after three or four generations.

Henderson [1931] in discussing the relationship of pica in cattle to trypanosomiasis has concluded that (a) Pica in Northern Nigeria bears a close relationship to trypanosomiasis of cattle, (b) the infection by trypanosomiasis causes an acidosis in animals with consequent upset of the 'alkali reserve' of the blood, (c) the excessive excretion of sodium and calcium during an acidosis cannot be compensated by the amount of these elements in the herbage normally grazed by cattle, (d) the uncompensated loss of sodium and calcium leads to a craving for salts containing these elements and it is expressed by the showing of pica symptoms.

Bisschop [1940] read a paper entitled 'Bionomic studies on indigenous and exogenous cattle in the semi-arid regions of the Union of South Africa' at the Seventh International Genetical Congress, Edinburgh in August 1939 and the suggestions for work under Indian conditions contained in this note are based largely on the work reported in his article.

B. India

Under-nutrition, owing to lack of sufficient fodder for animals in the country, is probably the most important limiting factor to the development of cattle in India. In addition to general lack of fodder, however, there are in some areas marked deficiencies of minerals, protein and certain vitamins in the fodder. It is known that in many districts, in this sub-continent, the soil is poor in phosphates. Thus in Bihar soils, Davis [1918] found that the total phosphorus percentage varied between 0.086 to 0.137 and the amount available from a trace to 0.002 compared with about 0.27 and 0.16 respectively in good soils. The report of the Department of Agriculture of Madras for 1918-19 states that the crying need for almost all crops in Southern India is phosphoric acid. This poverty of the soil in phosphorus affects not only the composition but also the bulk of the pasture. Deficiency of phosphorus in the soil is, therefore, probably an important factor, both in under-nutrition and in malnutrition. Davis [1917] attributes low milk yield of cows in Bihar to deficiency in phosphorus in the pastures, and McCarrison [1927] has stated that in Bihar and on the coast of Malabar mortality among cattle is high, sterility is common, and the milk yield of cows low. These conditions are associated with pica. McCarrison suspects the cause to be lack of phosphorus. Warth [1927] puts forward the idea that it is probable that cattle in India suffer from deficiency of minerals other than phosphorus and that there may be a deficiency of [common salt, i.e. of

sodium and chlorine. It is a well-known fact that lack of salt has an adverse effect on the health and productivity of cattle and that the requirements for salt are specially high in hot climates.

The extensive investigations of McCarrison [1913] have indicated that there is a shortage of iodine in areas in the region of the Himalayas, but so far as is known this shortage is not associated with high incidence of goitre and its sequelae in cattle, though these conditions are very prevalent in human beings in some of the Himalayan regions.

The economic importance of deficiency of minerals in the pasture was referred to by several witnesses giving evidence before the Royal Commission on Agriculture in India. Warth [1927] offered the opinion that it was impossible to lay too much stress on this subject. He believed shortage of calcium, phosphorus, sodium and chlorine occurred in various parts of the Empire and that deficiency of phosphorus was perhaps the most important Indian problem. McCarrison [1927] who has done exceedingly valuable work on deficiency diseases also emphasized the importance of deficiency of minerals, especially of phosphorus and iodine, in causing malnutrition which he regarded as the most far-reaching cause of disease in India. Oliver [1933] has stated that one of the fundamental causes of degeneration of stock and loss to stock-owners is defective nutrition due to ill-balance or actual deficiency of essential food ingredients.

Ware and Sen [1937] have indicated as a result of a survey of the problem of malnutrition carried out at the Imperial Veterinary Research Institute, Mukteswar, that the effect of these deficiencies is not confined to a general physical deterioration of the stock, their productive power has been affected, breeding difficulties have become more common and susceptibility to certain classes of disease has been increased. To give a few illustrations on the above point, mineral deficiency has given rise to pica and osteomalacia in Madras and Hyderabad and the stunted growth of animals in Berar has been ascribed to the same cause. This is probably also true in parts of Bengal, Bihar, and Assam. Milk yield in most of these areas is low, mortality is high and sterility is common. The disease known as 'blindness in calves' which is usually associated with a non-specific form of abortion in cattle has been shown to be due to malnutrition. It will thus be seen that the problem of malnutrition is an all-India one, but it may be found necessary to tackle this subject regionally because the type of malnutrition may not be the same in every part of the country. They have emphasized the necessity for investigations of a comprehensive nature to study the problem of animal nutrition in relation to main-

tenance of health, normal growth and increased productive capacity of animals in India. Ware [1937] has discussed the importance of grazing in regard to the development of cattle and dairying industries in India and has made appropriate suggestions for the extension and improvement of pastures in India. Valuable studies of Indian grasses and grasslands have been made by Burns, Kulkarni and Godbole [1925 and 1928] with special reference to the Province of Bombay having varied climatic conditions and consequently different kinds of grasslands and have made useful recommendations for the improvement and proper utilization of grasslands.

Lander [1937] has briefly summarized the grazing condition in the different provinces and has recorded the results of chemical analysis of common Indian fodders and grasses grown on different soils and under different geographical and climatic conditions and at various stages of growth. He has also given the results of mineral survey of the Punjab soils carried out by him. Referring to certain special areas which are noted for the richness of natural fodder and the excellence of animals which graze it, he has stated that the most notable of these is perhaps the Government Cattle Breeding Farm at Hissar comprising some 37,000 acres of natural grazing land, part of which is enclosed as irrigated paddocks. Hissar is the home of the famous breed of Hissar cattle which thrive to perfection on the highly nutritious grasses found in this area. The chief of these are (1) *Anjan* (*Pennisetum cenchroides*), (2) *Bur* (*Andropogon laniger*), (3) *Panni* (*Andropogon muricatus*), (4) *Palwan* (*Andropogon pertusus*) and (5) *Dub* (*Cenodon dactylon*). *Anjan* is the best grass on the *bir* (grazing ground); it grows over vast areas and is highly nutritious; it makes first class hay and has proved to be a most satisfactory ration.

Viswanath [1941] in his presidential address to the Section of Chemistry and Biology at the 10th Annual Meeting of the National Academy of Sciences has reviewed the recent developments in the Science of Plant and Animal Nutrition and their significance to national nutrition and health and has emphasized the importance and value of cattle manure for the growing of crops. It is unfortunate that at present cattle manure, an extremely valuable fertilizer, is used more as a fuel in the form of *uplas* than as a manure. His experiments carried out in collaboration with McCarrison [1927] and with Suryanarayana [1927] have shown that the grain from cattle-manured crops, which showed greater vigour and grew a better plant, also possessed better nutritive value and higher protein and vitamin content than the grain from the mineral-manured or unmanured crop. Organic manures, besides improving the physical condition

of the soil and being sources of the ordinary food constituents for plants, also supply some agents like auxinones or vitamins which contribute greatly to the growth and reproduction of plants. In addition to their bio-chemical activity, the micro-biological population of the soil contributed directly to the plant some stimulant which is ultimately passed on to the animal. It appears that auxinones for plants and vitamins for animals are probably the same, or if different, exist together and are independent, functioning in different ways, according to the organism in which they are introduced and the conditions under which they operate.

Sen [1933] has stated that a good breed will deteriorate if kept for a long time on a defective ration. He has referred to the harmful effects produced by the excess of fluorine in the diet of cattle in fluorine-rich areas, especially where fluor-spar deposits occur and has stated that this substance is found in India in certain parts of the Central Provinces (Raipur District) and in some places in the Punjab and Madras Presidency. He is of the opinion that deranged endocrine functions (e.g. thyroid, pituitary) are likely to be associated with unbalanced rations supplied to the animals. He has stated that a very curious fact is that most of the good animals in India come from the tracts where rainfall is low and water is scarcely available. With the increase in the irrigated areas and consequent increase of crop production, grazing areas are getting fewer in number and animals coming from irrigated tracts are much inferior in condition, so far as their performance is concerned, and, moreover, these animals are more susceptible to parasitic infestations and diseases in general. Experiments carried out by Roy and Sen [1933] at Mukteswar, which is situated at an average elevation of about 7,000 feet above sea-level in the Kumaun hills, show that with the growth of the plants and increase in rainfall, the percentages of lime and phosphorus in pasture grasses increase to a maximum in September-October. After this period, the amount of phosphorus decreases considerably with the approach of winter and dry conditions. No marked decrease in the lime content, however, takes place. They are of opinion that during the winter months, the ratio between lime and phosphorus may be abnormally high, and hence a ration composed mainly of a winter-cut hay without the addition of phosphorus-rich concentrates, may induce aphosphorosis and malnutrition in cattle. Sen [1935] has stated that deterioration seen in most of the indigenous breeds in India is due to a number of causes, of which malnutrition and disease are probably the most important causes and which are to a large measure inter-dependent. To a great extent indigenous animals

appear to have adapted themselves to the type of food-stuffs available in this country, namely to a diet containing a low amount of protein and minerals and a fairly high amount of fibre, but the net consequence of this malnutrition has been the survival of a type of animal which is small, undeveloped and economically very unproductive. The milk yield of the cow is low, breeding difficulties are common and in the case of draught animals, the return in terms of work is very small. Large numbers of such animals exist throughout India and are, from an economic standpoint, a serious loss to the country. Any attempt to improve the animal industries of India must, therefore, take into account the need for adequate and well-balanced diets for domesticated animals of all kinds, and in order to study which constitute a well-balanced diet, systematic research work on the nature of our food-stuffs is essential. He has also emphasized that there is an intimate connection between malnutrition in cattle and malnutrition in human beings. Sen and Seshan [1938] have described the various conditions, e.g., congenital blindness, night-blindness, abortion, lowered vitality, etc., associated with avitaminosis A in cattle and have suggested the ways and means of controlling these conditions in the field. The available sources of this vitamin or its precursor (carotene) for livestock are discussed and suitable methods of preserving green fodder with minimum loss of carotene are given.

Lander [1939] who made a comprehensive nutritional survey of the Kangra Valley has remarked in introducing his note on the correlation between soil deficiencies, poor cultivation, unthrifty cattle and human nutrition that not only were the cattle of this district of a small size but they give very little milk and a majority of these were dry during the greater part of the year. The main fodder of these cattle during the greater part of the year was rice straw, very rarely supplemented by concentrated food. It was deficient in protein, calcium and phosphates. This unsatisfactory system of agriculture led to insufficient supplies of animal products like milk to the inhabitants who, therefore, suffered from deficiency diseases like osteomalacia, goitre, etc. He stated that high rainfall involved considerable leaching from the soil of soluble minerals, the most conspicuous of which was calcium. He suggested that each province should endeavour to concentrate on most outstanding features of malnutrition in their territory and having found the causes should set to work to remedy them. Olver [1937] in discussing the relation of animal nutrition to public health in India has suggested that village cultivators and stock-owners should produce more fodder crops, more and better farmyard manure or compost and better stock, thereby increasing their income and

the nutrition of the family, while maintaining the fertility of their holdings and making a substantial contribution to the maintenance of public health.

Corrie [1937] has stated that the awful deterioration in the type of cattle wherever common grazing persists as the usual practice is a most striking and consistent feature of Indian animal husbandry. This deterioration is seen at its worst in all districts containing large areas of wild land, too steep or too poor for cultivation, and, therefore, includes the whole of the Western Himalayas and the many ranges of lower hills further south. In these common grazing areas almost every kind of livestock competes for a living and large migratory flocks of sheep and goats which descend from the high hills every autumn to the adjoining plains, like locusts in their number, cause sheer destruction of the available grazing. On the other hand, better-known breeds of Indian cattle are found in areas where there is practically no common grazing ground left, e.g. dry areas of the Punjab and where the people have been forced to rely mainly upon stall-feeding and to concentrate upon fewer and better animals. At the time when hay ought to be made in the months immediately following the monsoon, the cultivator is busy with his autumn ploughing, so the cutting of grass is inevitably delayed until only the dry bones of the grass crop are left. It is not possible to alter the agricultural calendar, so improvements appear to lie along the lines of grass cultivation by contour ridging and by any other means which keep the grass green for a longer period. Shah [1939] has stated that it is a well-recognized fact that the intense over-grazing to which the ravines and other grazing grounds in the United Provinces are subjected prevents the maximum production of fodder, and that the only way to increase the fodder supply from uncultivated lands is to reduce the incidence of grazing. In ravine areas, goats, in particular, are most destructive to the natural vegetation. The finest cattle in the Provinces are to be found in districts like Meerut where the grazing grounds are at a minimum and the worst cattle in districts such as Jhansi where the grazing grounds are at a maximum. It is of little use to spend money on breeding bulls and improved breeds of cattle, unless the improved breeds and their progeny are better fed. The only way to do this is to grow more fodder on cultivated lands and to reduce the intensity of grazing on uncultivated lands.

Aiyer and Kayasth [1931] have analysed the grasses and fodders growing in different parts of Central Provinces and Berar and have found that the grasses are very poor in phosphoric acid, calcium and nitrogen contents. The grasses grown on heavy black soil are, however, richer in mineral content than those grown on light soil. The poor stunted growth of the cattle in Chhattis-

garh may be due to the phosphate deficiency in the ration. They are of the opinion that the lime deficiency may not be so pronounced as phosphate deficiency, for *jowar*, *kudbi*, paddy straw and wheat straw which form a good part of the ration contain high proportion of lime. They have suggested that the addition of an oil-cake may make up the phosphate deficiency and the lime or nitrogen deficiency, if any, can be made up by the addition of leguminous fodders, e.g. berseem, *jowar*, soyabean *kulthi*, etc., in the ration. In the rice tract, the rice polishing (*krunda*) which is rich in phosphoric acid should not be wasted but utilized in feeding to the cattle. In the wheat tract *bhusa* of the leguminous crops should form a part of the ration to the cattle. Iyer [1935] has shown that the mineral composition of grasses may be affected by the type of the soil, the species of grasses, maturing and cultivation. Chaudhuri [1933] has reported that calves which were stunted in growth improved considerably in weight and condition after being fed a mineral supplement.

Bhalerao [1934] has stated that in India there are numbers of ailments of cattle which go unnoticed and undiagnosed and which, although not in all cases apparently serious, undermine the health gradually and render them incapable of serving humanity to their proper extent, while in some cases they even take their toll of life. Such diseases are usually caused through the agency of worms which are parasitic in the various organs of these animals. Although exact data are not available at present in regard to the loss that is actually caused by worms, it is certainly larger than generally imagined. The Veterinary Investigation Officers working under the Imperial Council of Agricultural Research schemes have discovered the existence of nutritional disorders, e.g. osteomalacia, pica, infertility, eye-troubles etc. and high incidence of parasite infestations in certain parts of India which include the notorious tracts referred to in this note. The Council's Helminthological schemes and the nutritional schemes have thrown and will throw in due course more light on these aspects of this vast problem of deterioration of cattle. Carbery, Chatterjee and Talapatra [1937] have carried out a fairly comprehensive series of experiments to determine the mineral requirements of cattle in the rice growing tracts in north-east India. The results indicate that *aus* straw is considerably superior to *aman* straw in minerals and protein and hence in general feeding values, but both straws are poor in phosphate and slightly deficient in lime. The phosphate might be conveniently supplied through cake while a small supplement of chalk would make up lime deficiency. Rice *kura* (bran) is very poor in CaO and unusually rich in phosphate (over 6 per cent). The CaO and chlorine figures have

definitely given a negative balance; while in spite of heavy P_2O_5 ingestion (39 to 59 gm.) a negative balance has been recorded up to the stage of 43 gm. This is probably due to the presence of phosphorus in the rice bran mainly as phytin which is not readily assimilable. Rice bran should, therefore, be supplemented with lime and chlorine on one side, while its phosphorus compound should be brought into assimilable form by hydrolysis. In the case of green feeds, Guinea grass is better balanced than Napier and Hyacinth, but the feeding should be regulated in order to avoid a large ingestion of lime. Napier grass should be supplemented with $CaCO_3$ and both Napier and Hyacinth should be so regulated as not to involve a larger ingestion of potash through them. They both (especially Hyacinth) require some cake supplement. Hyacinth is often used as a fodder in some parts of Bengal when there is a fodder scarcity.

Chatterjee [1942] has stated that in the rice-growing tracts rice straw has been and will probably remain the chief roughage and as its use must have largely contributed towards the present situation its judicious feeding is of much importance to the future well-being of cattle. The main deficiency with rice straw is that it is very poor in protein and phosphorus and contains a large quantity of crude fibre on one side and an interfering substance (oxalic acid) against a proper absorption of lime on the other. Again depending on the nature of feed combinations, the digestibility of straw protein varies from nil or even negative to 3, 4, 5 per cent going up to 37 per cent. When a balance sheet was drawn of the intake and outgo of the essential body components, more protein, lime, magnesium and phosphorus were drained out of the body than were supplied in the feed, with the inevitable result that the animal lost in condition, body weight and thriftiness. As soon as the animals were provided with a suitable concentrate (linseed cake) a valuable combination of protein and phosphorus ensued with the result that not only did the general appetite and consumption go up but there was definitely better utilization and assimilation of the food components and the animals gained in the weight.

Ramiah [1939] has stated that an area in the Kurnool district was investigated as a deficient area in phosphoric acid as revealed in the pasture survey. A disease commonly known as Vayn-Potlu resembling ostomalacia or arthritis is endemic in that area. Blood phosphorus was very low, giving a blood picture of pronounced imbalance. The presence of fluorine and mineral imbalance are factors operative in the incidence of the disease. A similar field survey of cattle in South Kanara district showed less pronounced symptoms of deficiency resulting in general poverty of condition

in cattle. Herbage protein studies revealed that manuring has a marked effect on the total nitrogen content and type of distribution of nitrogen in the herbage of cereals.

Anwar Ullah [1939] has stated that most of the constituents, especially the minerals in the fodders, tended to decrease with advancing age, the main exception being in the case of nitrogen-free extract which always increased with age. In certain cases, of course, this did not hold true which is mainly explained by some disturbing conditions, such as damages due to drought, flood, etc., or disturbing conditions like manuring, etc. Influence of soil conditions in Bihar was very marked and it may be said that all fodders grown at Sabour, Darbhanga, Pusa and Patna are expected to be rich in most constituents, especially the important ones like protein, calcium and phosphoric acid, whereas the fodders grown at Kanke, Jamui, Nawada and Banka are generally expected to be poorer. Exceptions, of course, exist but these show the general trend. Though there were no controlled experiments on the effect of seasons on the composition of fodders, yet it is observed in a general way that the fodders grown in the 1938 season were richer than the fodders grown in 1936 or 1937. In some cases the 1936 crop was also richer than the 1937 crop, which seem to have been the poorest in recent years.

SOME PRACTICAL SUGGESTIONS

To elucidate precisely the causes of degeneration of cattle in various parts of India is a difficult and complicated problem requiring systematic investigation and involving considerable expense and time. For such an investigation it is necessary that data should be collected from the affected areas regarding local environment, e.g., climatological factors, condition of soil and water, nutritional deficiency including that of important minerals like calcium and phosphorus or their imbalance and of vitamins, especially fat soluble vitamins, A, D and E, the excess or absence of trace elements like fluorine, cobalt, manganese, etc. and the incidence of diseases and parasitic infestations including blood parasites etc. The probable causes of deterioration in these areas should first be sorted out and the point settled whether the various suspected causative factors act singly or in combination by actual experimentation upon batches of local cattle and cattle imported from good areas where deterioration is not marked. The actual number of these batches will depend upon the number of suspected etiological factors, e.g., climate, diet, parasites, etc. and the possible combinations in which these factors may be operating, keeping suitable control.

In each experimental batch the unwanted factor or factors should be completely eliminated as far

as possible, e.g. by supplying the deficient mineral or vitamin or protein in adequate quantities by the most suitable route, by overcoming parasites by regular use of suitable drugs and examination of faeces or blood as the case may be, by dealing with the trace elements as required, and by regular immunization of the animals against the prevalent contagious diseases like rinderpest, haemorrhagic septicaemia, etc. Regular data in detail with photographic records should be collected from all the animals before the commencement of the experiment and during the course of the experiment and the beneficial results accruing therefrom in regard to growth, milk production, capacity for draught and reproduction should be observed. Chemical and biological analysis of blood, skin and hair studies and, where necessary, accurate clinical and detailed *post-mortem* examination should be carried out. This kind of investigation can be carried out successfully only if long-range experiments are planned, say for a period of at least ten years, and it should be remembered that such a comprehensive investigation may produce very important and far reaching results.

However, by a careful analysis of the available information on the subject, some general conclusion can be drawn with which it might be possible to take some steps immediately with a view to tackling this serious problem with some degree of success. The following points arising out of the review of the existing literature deserve careful consideration :

1. *Grow fodder crops.* Good cattle are found in comparatively drier parts of the country where people grow or are forced to grow fodder crops for the maintenance of their cattle in the absence of sufficient pastures. It seems, therefore, necessary that if cattle-wealth of the poorer areas is to be improved a suitable proportion of the land should be under fodder crops. To make the agriculture more remunerative and the cultivators healthier and more prosperous, it is essential that fewer cattle with greater production and capacity for work should be maintained and fed properly utilizing the limited fodder resources in the best possible way.

2. *Balance rice straw.* Rice straw is the staple cattle feed in the humid rice growing tracts of India where poor type of cattle with stunted growth, very low milk yield, stamina and capacity for work are generally found. Even good cattle imported from healthier areas tend to deteriorate in these areas in course of time. Rice straw is deficient in protein, phosphorus and vitamins and contains a large quantity of crude fibre on one hand and oxalic acid which interferes with the proper absorption of lime on the other. Knowing that protein is the body builder, calcium and phosphorus the bone former and vitamins essential for

wholesome assimilation of food and proper development and functioning of the body, it is not difficult to imagine what the cattle lose when they are fed mainly on rice straw. Under the Imperial Council of Agricultural Research animal nutrition scheme in Bengal, Indu Bhushan Chatterjee has shown that these defects in rice straw can be remedied by balancing it up with the addition of the locally available feeds such as oil-cakes (ensures valuable combination of protein and phosphorus), rice bran (contains protein of high biological value and is rich in oil and phosphorus*) and pulses (rich in proteins) in small quantities along with some green forage (especially to supply vitamins). Calcium and phosphorus supplement, e.g. a small quantity of powdered chalk and sterilized bone meal flour may be added to the diet when necessary. A fairly good sterile bone-meal flour can be prepared locally by the following method :

In an open vessel, meat-free and comparatively fresh bones may be cooked in boiling water for sufficiently long period till the bones become soft enough to disintegrate on beating. The broken chips of bones are again cooked twice, every time in fresh boiling water. The cooked bones are finally washed thoroughly in hot water. After allowing them to dry in the sun for two to three days, they are crushed into powder. This bone meal powder or flour prepared for feeding stock, should have but little odour, and should be nearly white in colour.

3. *Control of parasitic and bacterial diseases.* Humidity in the soil and air is more favourable for the development of parasites, especially helminths and the spread of soil-borne bacterial infections like Anthrax, Black-quarter, etc. Acute bacterial infections may cause heavy mortality, but losses from these diseases can be prevented by the use of appropriate sera and vaccines issued by the Imperial Veterinary Research Institute, Mukteswar. Regular vaccination of stock against these diseases in infected localities preferably before the onset of monsoons is desirable. Parasitic infestations both external and internal and chronic protozoan infections cause deterioration of the affected animals by adversely affecting their growth, milk yield and capacity for work, although in most cases they may not succumb for years. Regular administration of parasitocides against internal parasites and the use of cattle-dips against external parasites and the tick-borne protozoan infections should be extensively employed in these areas as a routine.

4. *Prevent soil erosion.* As a result of heavy rains the soil may be denuded of mineral matter and the fodder on such areas does not grow luxuriantly and does not contain enough of mineral

*It is not present in an assimilable form, but it can be rendered assimilable by hydrolysis.

constituents essential for proper animal growth, production and reproduction. Where necessary soil erosion may be prevented by resorting to bunding in the plains and by terracing and gully

trapping in the hills, etc.

5. *Improve pastures.* Pasture where available is usually the cheaper feed as well as one of the best for feeding either milch or draught cattle.

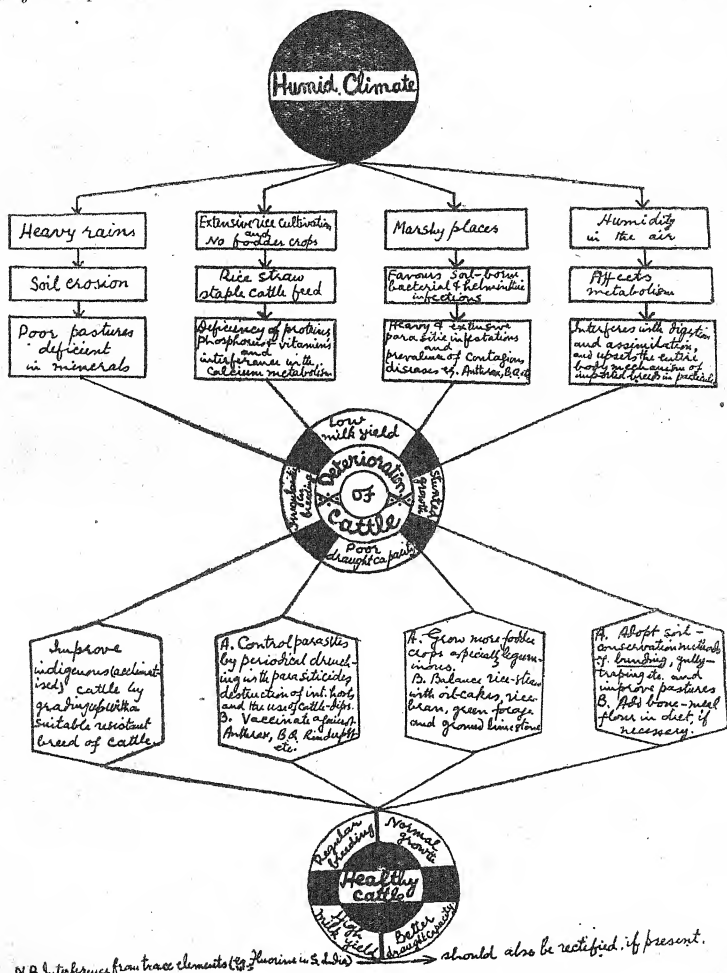


FIG. 3

Although in humid areas pasture lands are found here and there, yet on account of injudicious and overgrazing by much larger number of livestock than can be maintained on them and due to their ill management they do not serve the desired purpose. Not only the grazing areas should be extended by utilizing more of the land under the control of the Forest Department and that classified as 'cultivable waste', but what perhaps is more important is that the existing pastures should be properly developed and well managed. Improved varieties of grasses should be introduced, 'banding' may be made use of, rotational grazing, both in regard to pasture land and species of animal grazed, should be practised allowing restricted number of healthy livestock only, shady trees and drinking water should be made available, the land should be suitably dressed and manured where the soil and plant analysis indicates any deficiency, etc.

6. *Trace elements.* Where there is an indication that any of the beneficial trace elements e.g. copper, cobalt, manganese, etc. is lacking in the cattle feed, it should be provided in a suitable form. On the other hand, if there be evidence to believe that any of the harmful trace elements e.g. fluorine, selenium, etc., is present in intolerable quantities in the diet, necessary steps should be taken to get rid of such an element by the proper method. In the Province of Madras fluorosis has been shown to be the cause of rheumatic arthritis locally called 'Vayu-Potlu'. If drinking water contains 1.0 part or more of fluorine per million parts of water, it causes fluorosis. It has been found that the addition of lime removes the greater part of fluorine from water and renders it harmless.

7. *Resistant breeds.* There is some evidence to show that different breeds of cattle and buffaloes vary in their ability to stand the conditions prevailing in humid rice-growing tracts. Some are more susceptible than the others, though ultimately all might revert to something like the local cattle. Undoubtedly, the local cattle having adapted themselves, though degenerated, can stand these conditions best. Therefore, the best policy seems to be to improve the local cattle on one side by grading them up with a suitable breed possessing sufficient ability to resist the local environments and on the other by providing economically balanced food and freedom from parasitic and other diseases. Under a scheme to be financed by the Imperial Council of Agricultural Research it is proposed to study the effect of humid climate in rice-growing tracts on the four important breeds of cattle and buffaloes which are largely imported into such areas viz., Haryana, Sahiwal, Sindhi and Murrah, keeping suitable controls in a healthier area and providing similar food in similar quantities and freedom from parasitic and other diseases.

The results of this scheme will show the order of resistance amongst these breeds. Fig. 3 illustrates how deterioration of cattle is probably brought about in the humid areas and briefly indicates the methods to be adopted to remedy the same.

It is hoped that the above suggestions will be of some interest to those who are confronted with the problem and that they will collect systematically where possible such data as might indicate the suitability of certain recognized Indian breeds of cattle for different tracts in the country where cattle are deteriorated at present. Such data and those to be obtained in due course under the Imperial Council of Agricultural Research scheme might help the livestock officers, cattle owners and the Government in affected areas to plan a well organized scheme of work on a large scale in order to tackle this intricate and important problem effectively.

ACKNOWLEDGEMENTS

The author is greatly indebted to Mr F. Ware, C.I.E., F.R.C.V.S., I.V.S. Animal Husbandry Commissioner with the Government of India for valuable suggestions kindly given by him in the compilation of this note and to Dr F. C. Minett, D.Sc., M.R.C.V.S., Director, Imperial Veterinary Research Institute, Mukteswar, for the two maps kindly supplied by him to illustrate it.

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SELECTED ARTICLE

A PLEA FOR A BROADER SYSTEM OF VETERINARY EDUCATION, WITH SPECIAL REFERENCE TO INDIAN CONDITIONS

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AFTER completing thirty-five years in the Indian service one may perhaps be excused for indulging in a short review of the previous veterinary history of India and, what is more important, for making an attempt to plan for a better future. Incidentally, it may be observed that the present is a very opportune time for undertaking such a task, for when victory has been won there can be no doubt that within a short time India will at least attain a far more independent position amongst the United Nations than she holds at present, which will increase her already well-developed and natural desire to be self-contained in all domestic matters, including that of veterinary education.

In point of fact she has been more dependent on Britain in the past in the matter of higher grade veterinary education than in most other sciences, and this needs a word of explanation. Efforts have been made in India from time to time to introduce higher courses in veterinary education, on the lines of similar courses in the other sciences, but they have all foundered on the rock of finance. In dealing with a bureau-

cratic system of government the greatest disadvantage from which a profession can suffer is smallness in size, and this probably counts far more in a poor country like India than elsewhere. The demand for veterinary graduates of an advanced type has not been large enough in the past to justify the expense of setting up a separate college designed to turn out this type of man and India has had to wait until public opinion had been educated sufficiently to demand a higher standard of education amongst the rank and file of her veterinary profession and, incidentally, be prepared to pay these men for the extra time and money spent on their education, before a five-years' degree course could be introduced.

Even now the arrangement is not uniform throughout India. Graduates in veterinary science from the Madras University have already appeared, and arrangements are well advanced for the affiliation of the Lahore Veterinary College with the Punjab University, but in the case of the other three Veterinary Colleges at Bombay, Calcutta and Patna the matter is still under consideration. However, a good example is

usually followed in India, and one may expect in due course to see all the Indian veterinary colleges affiliated with the local University and turning out graduates to fill the more important posts in the Civil departments, their work being supplemented by 'Stockmen', a cheaper agency which can be trained in a few months to carry out much of the routine work for which a veterinary department is required in India today.

In a previous article which appeared in this journal for January, 1939, I endeavoured to sketch the early development of the veterinary profession and the establishment of veterinary colleges in India, and those who are interested are referred to that article for details. It is sufficient in the present connection to record the fact that there are 2,471 veterinary graduates in the employ of the Civil Governments in India today. Of these, eighty are Members of the Royal College of Veterinary Surgeons, England. These men are employed on all types of work, ranging from curative work in veterinary hospitals to those more important duties which may be included in the comprehensive title of Animal Health. This number constitutes the majority of veterinary graduates in the country, but by no means exhausts the list, for others are employed in the Defence Department, local bodies, private practice, etc.

In numbers, therefore, the veterinary profession in India is no mean part of the total Empire services, but even then they are quite inadequate for the prodigious number of animals for whose health and improvement they should be responsible. Apart from other animals, of which there are many millions, there are over 200,000,000 cattle in India, about one-fourth of the total cattle population of the world. Those who have visited India will say: 'What cattle! One could very well do without many of them.' This is true in part, but recent investigations have shown that if India is to cultivate her land properly and have sufficient milk for her women and children, she will require about that number of cattle. But they must be better cattle, and that is the major problem before Indian Veterinary Departments of the future.

How can productivity of India's cattle, and other animals, be increased within a reasonable space of time? It certainly cannot be done by the disjointed methods that have been adopted in many parts of India in the past. A real punch is required, and to get such a punch it is essential that there should be livestock departments (call them what you will) in each province, in which all aspects of the subject are closely coordinated by a master mind at the top. It cannot be sufficiently emphasized that

livestock improvement will not succeed without the help of the veterinarian, and the veterinary profession must identify itself very closely with livestock improvement and increased production, if it is going to survive as a separate entity, at least in a country like India.

It may well be asked what the veterinary profession in India has been doing all these years not to have seen that such an obvious arrangement was made for the development of the livestock of the country. Thirty-five years ago when one made an application for a post in the Indian service one was supplied with a memorandum which stated, amongst other things, that one might be employed on administrative work, teaching duties, or horse—and cattle-breeding. By the time that one arrived in the country, however, all the important horse-breeding areas had been removed from the control of the Civil Veterinary Department, and a few years later cattle-breeding in most of the provinces followed suit. There was, of course, plenty of other work for veterinary officers to do; in particular, the ravages of rinderpest in most provinces were of great concern, but this disease is, fortunately, no longer the menace to livestock improvement that it used to be, thanks to the improved methods of vaccination now employed.

The main plank in the argument of those interested in removing cattle-breeding from the control of the Veterinary Department was that improvement of stock in India was almost entirely a matter of better feeding, and a number of centres were set up where feeding trials were introduced, from the results of which we are benefiting considerably today. It is, however, now gradually being realized that animal nutrition is only one of the items to be tackled in order to effect permanent livestock improvement and, moreover, to get the best results in animal nutrition work one requires men whose main subject has been animal physiology.

This is the crux of the matter. When research workers in animal physiology were required, they were not forthcoming from the veterinary profession and they had to be found elsewhere. Today, when animal geneticists are required, there is the same story to tell. Many of the advances made in veterinary helminthology, entomology, protozoology and even bacteriology have been through the efforts of members of the medical profession and pure scientists. There can be only one reason for this and that is the system of veterinary education prevalent in the past in British veterinary colleges, which have catered largely for the practitioner class and

the Army. These absorbed the bulk of the graduates and on the whole the system will have been considered to have satisfied requirements, but as a training for potential research workers and for those called upon to deal with the health of livestock in the mass, which is the lot of most of those who elect to serve outside the British Isles, it was sadly lacking in several respects. This in itself might not have been very serious, for being recruited largely from the stock-owning classes British veterinary surgeons were soon able to adapt themselves to the changed conditions, but unfortunately the tradition has been passed on to India, where the training of veterinary graduates in the past has followed very closely the British system.

How can the position be remedied? The departmental Committee which considered the subject of veterinary education in the British Isles a few years ago made a number of very sound recommendations for its improvement and it is most unfortunate that it has not been possible to give effect to any of these during the war, for every year that passes provides us with a batch of new graduates who appear, to those working abroad, to be lacking in certain essentials of their profession. We require men whose training has been woven round the subject of animal physiology and have practical experience of animals in health and production, as well as in disease. A veterinary student cannot spend too much time on physiology. Not only does this subject form the best stepping-stone to many branches of veterinary science in which a man may wish to specialize, e.g., pathology, immunology, dietetics, genetics, pharmacology and up-to-date clinical medicine,

but no other subject in the veterinary curriculum will do so much to broaden a student's mind and increase the imagination, in which so many veterinary graduates, who have been trained in short cuts for passing examinations, increasing cash returns or swelling the out-patients register, are wanting. We require men whose conception of preventive medicine does not begin with the use of a hypodermic syringe after a disease appears in a village, but with the selection of the correct types of breeding-stock and the care of the animal organism from the stage of fertilization of the ovum onwards.

I shall, no doubt, be accused by some of joining those who delight in having a tilt at the young graduate, but that is not my intention. I feel that he has a good deal to complain of and in recent years I have noticed a distinct tendency on the part of veterinary students themselves to ask for a more imaginative course of professional training, which is a very healthy sign. In India, where veterinary education is at the moment much to the fore and new curricula are being devised, a great opportunity presents itself, and it is to be hoped that those in authority will see that old traditions are no longer blindly followed and that, at least in the degree courses, the system of veterinary education introduced will provide the necessary basic training to enable a graduate to specialize in any branch of science pertaining to animals which he may select, and ensure his acceptance on an equality with veterinary graduates of other countries in the administration of international conventions and other measures designed for the improvement of animal health and animal production.

ABSTRACTS

Some new methods for studying intestinal amoebae and other protozoa. DOBELL, CLIFFORD (1942). *Parasitology*, 34, 101-12.

In this article the author has described the method of obtaining; (1) permanent preparations of intestinal amoebae from cultures, in natural attitudes, and (2) a new and simple method for staining amoebae and other protozoa. As a result of his experience for the last 17 years, he has found that, if amoebae are desired to be fixed in their natural forms, the technique which he recommends is very useful. (Original article should be consulted for minute details). Briefly, the method consists of introducing aseptically thin cover glasses (No. 0, not exceeding 0.120 mm. in thickness and 7/8 in. square), which have been bisected somewhat obliquely, into the culture medium prior to the inoculum is added. Such cover glasses can also be inserted in growing cultures of any required age. In

the first instance, the inoculum is to be deposited at the bottom of the tube and on the upper surface of cover glass; in the second, the sediment at the bottom of the growing culture is to be sucked up by means of a sterile pipette and then re-distributed in the same manner. The culture tubes are to be incubated in a slanting position. Amoebae in such a culture tube creep on the surface of the cover glass and can be removed for study by means of a sterile platinum hook. In order to obtain a stained preparation of such a specimen, the cover glass showing numerous amoebae (film downwards) is dropped into the fixing solution contained in a hollow-ground glass block for a few minutes (5-10 minutes or longer if required). It is recommended that, after preliminary fixation, the cover glass should be turned to bring the film-side up and the surface squirted with the fixative in order to wash away a large number of bacteria, after which the cover glass may be dropped back into the fixative

for the desired period. The following staining methods have given him excellent results.

(a) *Tungstic haematoxylin method*

It consists of mordanting the film in a 2 per cent solution of phosphotungstic acid in distilled water for not less than 10 minutes. This period may be extended to several hours. Shorter period is said to be adequate for amoebae while the longer are better for cysts or flagellates. The film is next thoroughly washed in distilled water and stained for 10 minutes or longer (15-30 minutes or even several hours) in 0.2 per cent solution of "ripened" haematoxylin. Cysts and flagellates require a longer time for staining. After staining, wash in distilled water and then transfer to tap-water where it may be kept for 15 to 30 minutes (the water may be changed 2 or 3 times). The film appears purple to the naked eye and is ready for dehydration with alcohol, and is ultimately mounted in balsam or damar in the usual way.

(b) *Molybdic haematoxylin method*

This method is exactly like the foregoing, except that a 2 per cent. solution of ammonium molybdate is employed as a mordant in place of phosphotungstic acid. The author has obtained equally good preparations of *Giardia*, *Entomozoon*, *Embadomonas*, etc., with both methods of staining. Advantages of these methods are that they are rapid and easy, and do not involve the process of differentiation under the microscope, as is customary with ordinary iron-haematoxylin method. [H. N. R.]

The storage of artificially dried grass, Wright Normanc (1942). *J. Agric. Sci.* 31, 194-212.

The paper deals with the effects of humidity and moisture content—the two generally recognized important storage deterioration factors—on the keeping quality of artificially dried grass. Certain drawbacks in the drying processes and storage are pointed out.

Preliminary experiments over humidity ranges of 40 to 100 per cent revealed a close parallelism between humidity and moisture content. Detailed experiments based on the above findings under accurately controlled conditions revealed an extremely high moisture content of dried grass in atmospheres of high humidity; thus at 80 per cent humidity the moisture content was 18 per cent, at 90 it was 28 per cent and at 100 per cent humidity it was 50 per cent. Differences in composition did not materially affect the humidity-moisture content relation.

Tentative observations spread over 18 months on the level of humidity and moisture content favouring deterioration revealed that the critical humidity for mould growth lay between 67 to 80 per cent. Extended observation over these ranges shew (a) that the mould growth was a definite function of relative humidity i.e., in three days at 100 per cent relative humidity; five days at 90 per cent; 50 days at 75 per cent and in 300 days at 70 per cent humidity, (b) a contradiction of the usual assumption that if mould growth did not occur in two to three weeks, the product was immune from deterioration, emphasizing the need for a prolonged study, (c) that mould growth may take place even at 70 per cent relative humidity if stored for a long time.

Experiments designed to detect mould growth at an earlier stage by plating methods rather than by direct mycelial detection under the microscope proved unsuccessful. The rate of increase in mould growth was dependent upon moisture content and humidity and for reasonably safe storage, a relative humidity

of 67 per cent corresponding to a moisture content of 13 per cent was recommended, though this does not confer absolute immunity from mould growth. At a given moisture content, mycelium formation was as a rule seen to precede the development of musty smell while the latter intum was capable of being detected long before a significant rise in plate count could be made out; as such, the first two were considered to be the earliest and most reliable indications of mould growth. Caution was indicated in recommending limits of moisture content for long period storage, as this is dependent upon the mould microflora, hygroscopic nature of sample and local changes in moisture content and therefore in the surrounding air humidity of the sample. [K. G. R.]

Cystic pituitary in young cattle with vitamin A deficiency. L. L. MADSEN, S. R. HALL and H. T. CONVERSE (1942). *J. Nutr.* 24, 4.

DURING their studies on the gonadotropic and lactogenic hormones of the anterior lobe of the pituitary, the authors examined more than 10,000 pituitaries from apparently normal cattle and observed that only four or five glands in the whole group were definitely cystic. In contrast to the above observation, in a planned experiment with young cattle which developed varying degrees of vitamin A deficiency, these authors could demonstrate a large occurrence of cystic pituitary gland. For the purpose of the experiment two groups of animals were chosen, one consisted of beef animals of shorthorn and shorthorn-herford cross, the other group belonged to dairy Jersey and Holsteins.

Amongst the beef animals, two were depleted of vitamin A by continuously feeding a deficient ration for sometime. On autopsy, the pituitary glands of both were found cystic. In one of these animals, which was on a vitamin deficient diet early in life but later fed adequate amounts of carotene, no evidence of repair in the cystic pituitary was found. Apparently the injury to the gland brought about by vitamin A deficiency at the early part of life was permanent. The other beef calves were from cows receiving 30 to 45 Ug. of carotene per kilogram of body weight. Symptoms of vitamin A deficiency were evident in these calves at birth. All of them except one had cystic pituitaries.

The dairy animals examined in this study were all born to cows receiving sufficient carotene for normal reproduction. Soon after their birth, these animals were kept on rations of varying degrees of vitamin A deficiency. In due course, all the animals developed symptoms of deficiency. Two of the animals receiving the least carotene had very large pituitary cysts. Two others receiving apparently adequate amount of carotene developed cystic pituitary when carotene supplement was discontinued or partially withdrawn. In two other animals while the symptoms of vitamin deficiency were marked, the cyst in the pituitary of one was not large and histologically no evidence of injury to the glandular parenchyma was seen, while in the other the autopsy revealed an apparently normal pituitary.

The cyst in the pituitary of the vitamin A deficient animals occurred either in the residual lumen or within the posterior lobe, often causing compression of the gland and injury to the glandular parenchyma. According to the authors, the injury to the pituitary gland in calves under vitamin A deficiency was probably due to the results of the mechanism responsible for injury to both optic nerve tracts and other part of the central nervous system. [S. K. T.]

ORIGINAL ARTICLES

EXPERIMENTS ON THE TRANSMISSION OF ANTHRAX THROUGH FLIES

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(With Plate XII)

The experiments described in this paper were carried out at Mukteswar (alt. 7,500 ft.) between May and October, 1941-43. The daily maximum temperature range during this period was 68°-80° F. in May-June, 64°-72° F. in July-September and 64°-69° F. in October. Goats were used as experimental animals, since they are cheaper and more susceptible than cattle to experimental inoculation. The anthrax culture used was highly virulent for goats and gave typical rough colonies on agar. The species of insects tested were *Stomoxys calcitrans*, *Musca domestica*, *Calliphora erythrocephala* and, in one instance, *Sarcophaga* sp. In the earlier experiments, Tabanids (mainly *Tabanus orientis*) were employed, but, as none of these hill species would readily bite in captivity, the experiments were discontinued shortly after their commencement, with a view to resuming them in the plains where several species of Tabanids with better biting propensities were known to occur. It is hoped to report later on some of these plains species.

Although Tabanids are believed to be more commonly involved in the spread of anthrax [Mitzmain, 1914; Kehoe, 1917; Frey, 1919; Morris, 1920; Nieschulz, 1928; Olsufev and Leler, 1935; Kraneveld and Djaenodjin, 1940], several workers have also incriminated *S. calcitrans* as a transmitting agent. Thus, Mitzmain [1914] succeeded in transmitting anthrax from a guinea-pig to other guinea-pigs through the bites of *S. calcitrans* by the interrupted method of feeding, the flies being fed on the donor from 2½ hours to a few minutes before its death. Schenberg and Kuhn [cited by Schenberg and Böing, 1914] report having successfully transmitted anthrax to guinea-pigs and mice through *S. calcitrans*, while Schenberg and Böing [1914], after failing with one goat, transmitted the disease to one out of three sheep through the same species of fly. They point out that biting flies may be a much more important factor in transmission in some lands, e.g. N. and S. America, than in others, e.g. Great Britain and Germany. Hetsch [1915] likewise considers *S. calcitrans* to be the 'most dangerous carrier of anthrax', being able to carry the infection for about two hours and over a distance of not less than 1½ miles. Morris [1919], working with guinea-pigs, found *S. calcitrans* capable of transmitting anthrax mechanically from opened car-

buncular swellings of animals suffering from anthrax. He considers the fly may sometimes play this role in nature. He [1918] also records having obtained positive results by using the allied species, *Lyperosia (Haematobia) irritans*. Flies of this species were fed for one minute on guinea-pigs, previously inoculated subcutaneously with anthrax spores, and then on healthy ones from one to three minutes, the feeding being done through wire-gauze fixed on the top of a wooden box in which the flies were confined. The greatest proportion of successful transmissions was obtained when the infected animal was bitten a short time before its death. Also, an immediate transfer of flies from the infected to healthy animal appeared to be necessary for positive results. Morris refers to the bacilli carried on the proboscis of the fly producing, as a rule, the external or carbuncular form of anthrax. It is noteworthy that, according to him, the disease is not 'usually' spread through the excreta of infected insects and that *L. irritans* would not feed satisfactorily on anthrax carcasses. In contrast with the positive result obtained by Morris with *L. irritans*, Nieschulz [1928] records having failed to transmit anthrax through the bites of *L. exigua*.

As regards *M. domestica*, this species has been shown by Morris [1919] to be capable of carrying anthrax bacilli from anthrax-infected flesh and so contaminating wounds on healthy guinea-pigs. Nieschulz [1928] used *M. inferior* and obtained positive results with guinea-pigs in one out of six experiments.

The possibility of Calliphorids being concerned in the spread of anthrax has been mentioned by a number of workers. Dunn [1918] refers to the common phenomenon of swarms of screw-worm flies (*Chrysomya macellaria*) being attracted to blood-stained exudation from the mouths and nostrils of anthrax carcasses, and he, therefore considers it probable that some of these flies would infect any animal they might visit shortly afterwards, provided it has some unhealed skin abrasion. Osins' kii [1938] refers to the habit of *Lucilia* and *Sarcophaga* of sucking fluids from carcasses, e.g. of rabbits dead of anthrax. He describes experiments in which *Calliphora* and *Lucilia* flies were placed on agar, after the had sucked the juices from anthrax meat. In such cases, anthrax bacilli were found to 1

deposited on the agar. Morris [1919, 1922] demonstrated the transference of anthrax bacilli from anthrax-infected flesh to wounds on healthy guinea-pigs through the agency of *Calliphora erythrocephala* and *Lucilia* spp.

In the present experiments, the question of *S. calcitrans* being involved in the spread of anthrax is claimed to have been investigated in all its aspects, for these experiments not only included the feeding of infected flies on healthy goats, but also the testing of infectivity of faeces of such flies when deposited on the scarified or cauterized skin of goats. The experiments with *M. domestica* and *C. erythrocephala* were carried out to confirm the statements made by previous workers that these and allied species are capable of carrying the infection from anthrax-infected flesh to abrasions on the skin of healthy animals. A few experiments were also carried out to test the possibility of the disease being reproduced through contact of infected *M. domestica* with the eyes of healthy goats. As control to some of the experiments, a study was made of the effects of the direct application of anthrax spore culture to the normal intact skin of goats and also to skin treated in different ways. Two experiments were carried out to test the infectivity of *C. erythrocephala* bred from larvae fed on an anthrax meat.

Specimens from flies used in some of the experiments were bacteriologically examined for the presence of anthrax bacilli in their mouth-parts or bodies or in both, and in a few instances faeces of *Stomoxys* flies were similarly examined at various intervals, after their feed on anthrax-infected goats.

METHODS AND MATERIAL

Entomological

In the experiments with Tabanids, the latter were liberated in a fly-proof shed (Plate XII, figs. 1 and 2) measuring about $12 \times 6\frac{1}{2}$ feet and divided into two compartments, in one of which was kept the donor (anthrax-infected) goat and in the other the receptor (healthy) goat. The shed was covered with mosquito netting of 22 meshes to the linear inch and provided with double doors at both ends, in order effectively to prevent escape of the flies. In the middle of the shed, there was an adjustable door for admitting the flies, after their infective feed, from the 'infected' to the 'healthy' compartment. In no instance, however, were any of the flies actually observed to feed on the experimental animals, though they were provided with all possible inducement to do so, including a plentiful supply of moisture and vegetation. It is noteworthy that Mitzmain [1913, 2], in the Philippines, has recorded similar

experience with Tabanids in his experiments on the transmission of surra. He observes: 'An effort was made to induce flies [*Tabanus striatus*] to feed on healthy and infected animals kept together in a large screened cage. The results were negative, the flies dying in a few days when kept within the enclosure'. In the present experiments, recourse was, therefore, eventually had to feeding the Tabanids in tubes, but, as already stated, their natural disinclination to feed in captivity caused these experiments to be discontinued.

In experiments with *S. calcitrans*, *M. domestica* and *Calliphora erythrocephala*, three types of cages were used: (1) The usual type of cage consisting of a wooden frame and covered on all six sides with mosquito netting, with a hole and cotton sleeves on one side for the introduction of the arm; two sizes of this cage were used, one for holding about 60 and the other 200-300 flies. (2) The same type as above, but the whole of the mosquito netting on one side was replaced by a sliding metal door to enable the flies to be brought in contact with the body of the animal, without the intervention of the netting. This type of cage was used in the majority of the experiments with *M. domestica* and *C. erythrocephala* and will be referred to as the 'scarification cage.' (3) Cone-shaped cage covered with mosquito netting and designed for bringing infected *M. domestica* in contact with eyes of healthy goats; it was open at both ends, the narrow end being provided with an adjustable shutter and adapted for being placed over the eye of the animal. This type of cage will be referred to as the 'eye cage'.

Experiments with *S. calcitrans* were all conducted with wild specimens, and they were fed either singly in tubes or *en masse* in cages of the first type described above. The methods adopted for keeping the flies alive between feeds and for determining whether a particular fly has actually fed or not were essentially the same as those described by Sen and Abdus Salam [1937].

Specimens of *M. domestica* used in the experiments were collected from cattle sheds in the station, but in the case of *C. erythrocephala*, only laboratory-bred specimens were employed. Specimens of both these species were kept alive in cages by providing them with sugar solution to feed upon and enough moisture in the form of thick pads of cotton-wool saturated with water and placed on the top of the cages.

Bacteriological

In all cases where living donors were used the presence of anthrax bacilli in the peripheral circulation had already been demonstrated before feeding of the flies commenced. Usually a

separate cultural examination of the flies' heads and bodies was made, the heads having been removed immediately after the flies were killed. The wings were also removed. It was advantageous to 'clean' the material in the following way: it was first well shaken up in ten changes of sterile distilled water, drained as much as possible, immersed in absolute alcohol for five to ten minutes with occasional shaking, and dried on absorbent paper. The dried material was then powdered in a mortar and suspended in saline (2.5–10.0 c.c.) from which serial tenfold dilutions were made for plating on agar. Faecal spots, deposited by the flies on Petri dishes, were likewise suspended in 2.5–5.0 c.c. saline. After 24 hours at 37°C. anthrax colonies can be recognized at a glance if they are numerous, and there is little contamination. They can still be recognized with fair certainty, even if there are considerable numbers of discretely-growing contaminants. When there is extensive contamination of the spreading type, the anthrax growth is masked. When necessary, colonies are picked and injected to white mice for identification.

The numbers of anthrax bacilli in the bodies of *Stomoxys* flies after feeding on the infected goats varied considerably. In 12 instances, the numbers of colonies estimated to have developed from a single fly ranged from 1,250 to 1.2 million; in 10 of the 12 instances, the range was from 15,000 to 142,000.

TRANSMISSION EXPERIMENTS

1. Experiments with *Stomoxys calcitrans*

1. *Feeding infected flies on normal skin* (Table I). The flies were fed on anthrax-infected goats for varying lengths of time and then fed on healthy goats after intervals ranging from 0 to 48 hours. The main object of these experiments was to test the possibility of the disease being transmitted by the so-called 'interrupted' method of feeding, i.e. by interrupting the flies while engaged in feeding on the donor and allowing them to complete their feed on the receptor. In each case, the feeding on the donor was done only when anthrax organisms were actually present in the circulating blood. (The time elapsing between the finding of anthrax bacilli in the blood and the death of the animal was frequently five to eight hours or even more, so there was plenty of time for the flies to feed). In a few instances, the flies were allowed to feed completely on the donor, and in others, carcasses were used as donors. Mitzmain [1913, 1] has given 2½ minutes as the average length of time taken by *S. calcitrans* to obtain a full meal of blood from goats. Experience at Mukteswar has shown that the duration

of feed of individual flies is very variable, depending on the condition of the goat and of the fly itself and also on the temperature of the atmosphere. When the flies are being fed *en masse*, some allowance has also to be made for the fact that all the flies of a lot will not settle on the host at the same time, whilst individual flies are liable to be interrupted in the feed by the movements of other flies of the lot.

2. *Feeding infected flies on scarified or cauterized skin* (Table II). The object of these experiments was to test the possibility of the disease being produced by infected flies biting on fresh wounds or abrasions on animals. The flies were confined in 'scarification cages', fed on incisions made with a scalpel on the flanks of goats dead of anthrax and then transferred to the cauterized skin of healthy goats. The incisions were about three inches in length and one inch in depth. The cauterization was done by applying a heated spatula to the skin of the animal and then removing the epidermis of the scalded area by the blade of a scalpel, care being taken to avoid bleeding in the process. In the earlier experiments, the skin was scarified by making a series of superficial cuts on it by means of a scalpel, but this did not prove satisfactory owing to bleeding and hardening of the scarified area that followed.

3. *Causing infected flies to defaecate on scarified or cauterized skin* (Table III). In some instances faeces of infected flies, when tested culturally, showed the presence of anthrax organisms. This led to a series of experiments to find out if under natural conditions defaecation by *Stomoxys* on abrasions or wounds was likely to produce the disease. For the purpose of these experiments, the infected flies were confined in 'scarification cages' and brought in contact with the cauterized skin of healthy goats. At the end of the feeding period, some dozens of faecal spots could be counted on the exposed area of the skin. The results were negative.

In connection with these experiments, it was demonstrated that, as expected, anthrax can be set up in goats by lightly applying culture suspension to the freshly-cauterized skins, but not to skin from which hair has been carefully removed by scissors. Also, as expected, infection is produced when culture suspension is lightly applied to an area of skin, after removal of hair by scissors and after hungry *Stomoxys* have been allowed to feed on the area for 30 minutes or so. The bites rapidly cause an oedematous thickening of the skin, with bleeding points.

II. Experiments with *Musca domestica*

1. *Bringing infected flies in contact with cauterized skin* (Table II). These experiments were

Transmission of Anthrax through Flies

TABLE I

Stomoxys calcitrans feeding on normal skin

No. of living goats used as donor	Date of death,	Number of flies	Duration of exposure (minutes) on		Interval between exposures	Result	Remarks
			donor	receptor			
	1941						
110	July 25 1 P.M.	25	5	10	3 min.	Neg.	Same receptor goat used for these three donors. With donor 113 and the receptor flies were fed singly.
69	July 25 2-30 P.M.	25	5	10	3 min.	Neg.	
113	July 25-26 night	17	(Fed partial)	(Fed completed)	1 min.	Neg.	
116	...	50	2	3 to 5	3 min.	Neg.	
165	Aug. 6 6 P.M.	150	(Fed partial)	(Fed completed)	2 min.	Neg.	Footnote 1
165	...	150	15	15 15 42 hr.	15 hr. 21 hr. 42 hr.	Neg.	
203	Aug. 14-15 night	100	60	15 10 10	30 min. 18 hr. 23 hr.	Neg.	
205	Aug. 14-15 night	100	45	30 15 10	2 min. 18 hr. 23 hr.	Neg.	
64	Aug. 27-28 night	120	6	15 15 15	2 min. 17 hr. 23 hr.	Neg.	
64	...	120	48	15 15 15	2 min. 19 hr. 28 hr.	Neg.	
157	Sept. 12 2 P.M.	120	2	15	Nil	Neg.	
157	...	120	4	15	Nil	Neg.	
215	Oct. 4-5 night	120	2	12	Nil 24 hr.	Neg.	
215	...	120	6	12	Nil 24 hr.	Neg.	
	1942						
543	May 1-2 night	30	5	20	Nil	Neg.	Footnote 2
543	...	30	10	20	Nil	Neg.	
611	May 2 10-30 A.M.	30	5	20	Nil	Neg.	
611	...	30	10	20	Nil	Neg.	
6	Aug. 17 10-30 A.M.	50	15-20	Till engorged	Nil	Neg.	Fed singly. Do.
9	Sept. 22	15	30	do.	Nil	Neg.	
14	Sept. 30 11 A.M.	18	6	do.	Nil	Neg.	

1. With goats 165, 203, 205 and 64 the flies were fed once on the donor and thrice on the receptor as shown.

2. Four separate batches of 30 flies were used, the same donor being used for feeding two of the batches, which were then placed on one receptor.

3. All receptor goats, except that from donor 203, later proved to be susceptible to anthrax.

carried out on the same lines as those with *S. calcitrans*.

2. *Bringing infected flies in contact with the eyes* (Table IV). The flies were fed on incisions as above and then brought in contact with the eyes of healthy goats. In the earlier experiments, they were enclosed in cigarette tins or in 'scarification cages'. As neither of these proved satisfactory, use was later made of 'eye cages' described earlier in this paper.

All these results were negative, as might perhaps have been anticipated in view of the work of Aitoff [1922]. As a further check, two goats were given on the conjunctiva a suspension of anthrax from an 18-hour agar culture, but no infection ensued. The suspension was held in contact with the cornea and conjunctiva of one eye for several minutes before the animal was released.

III. Experiments with *Calliphora erythrocephala* and *Sarcophaga* sp.

1. Bringing infected flies in contact with cauterized skin. These experiments were carried out on the same lines as those with *S. calcitrans* (Table II).

2. Two experiments were carried out to test the infectivity of *C. erythrocephala*: flies bred from larvae reared for about 8-14 days from time of emergence from egg on meat from anthrax carcasses. The flies were then brought into contact with the cauterized skin of goats, for $1\frac{1}{2}$ hr. on each of two successive days in the first experiment and for $1\frac{1}{2}$ hr. on one day in the second experiment. The results were negative, and anthrax organisms could not be recovered on bacteriological examination of the flies or of larvae which were about to pupate.

Morris [1919] found that flies (*Calliphoridae* and *Lucilia*) bred out of unopened anthrax carcasses during the warm season do not carry infection, but that flies bred in the presence of anthrax spores are capable of doing so. It is of interest also that Graham-Smith [1911] found that many blowflies (*C. erythrocephala* and *Lucilia caesar*) which emerged from larvae fed for seven days on meat infected with anthrax spores were infected with *B. anthracis* and remained infected for 15 days or more. The faeces deposited by such flies contained considerable numbers of *B. anthracis* for at least two days. On the other hand, flies which emerged from larvae fed on the body of a guinea-pig dead of anthrax, that is containing only spore-free forms of the organism, were not infected with anthrax [Graham-Smith, 1912].

TABLE II

Transmission from carcass wounds to scarified skin

Carcase as donor from goat No.	Date of death	Number of flies	Duration of exposure on		Interval between two ex- posures	Result	Remarks
			donor	receptor			
<i>Stomoxys calcitrans</i>							
28	1942 June 15 6-15 A.M.	200	2 hr.	1½ hr.	Nil	Neg.	Flies were fed on clipped area on donor Flies fed on cau- terized, pricked and swabbed area on donor.
85	June 18-19 night.	100	2 hr.	45 min.	15 min.	Neg.	
76	June 12 10-25 A.M.	60	2 hr.	45 min.	30 min.	Neg.	
<i>Musca domestica</i>							
88	June 22 2 P.M.	50	30 min.	30 min.	Nil	Pos.	
28	June 15 6-15 A.M.	30	2 hr.	1 hr.	Nil	Pos.	
25	June 29	24	6 min.	6 min.	Nil	Pos.	
		24	6 min.	6 min.	Nil	Neg.	
1	July 6	24	15 min.	6 min.	Nil	Pos.	
		24	15 min.	6 min.	Nil	Pos.	
5	July 23	24	25 min.	6 min.	Nil	Neg.	
		24	25 min.	6 min.	Nil	Pos.	
<i>Calliphora erythrocephala</i> and <i>Sarcophaga</i> sp.							
80	Aug. 6 8-40 A.M.	40 Cal.	1½ hr.	1 hr.	Nil	Pos.	Footnote 1
610	Aug. 6-7 night	40 Cal.	1 hr.	1 hr.	Nil	Pos.	Footnote 2
137	Aug. 11 10-30 A.M.	40 Cal.	1 hr.	1 hr.	15 min.	Neg.	
6	Aug. 24	50 Sarc.	1 hr	1 hr.	15 min.	Neg.	

1 The same batch of flies was used for feeding on carcasses 80 and 610 and then on one receptor goat, first on one side of its body and next day on its other side.

2 One goat was used as receptor, first for carcass 137 and later for carcass 6. One side of receptor's body was used for the first feed and other side for the second feed.

3 With one exception (receptor from carcass 28), all receptor goats later proved to be susceptible to anthrax.

Transmission of Anthrax through Flies

TABLE III

Stomoxys calcitrans. Attempted transmission by faeces to scarified skin

Number of goat used as donor	Date of death	Number of flies	Number of goat used as receptor	Duration of exposure (hr.) on		Result
				donor	receptor	
581	1942 June 1 2 P.M.	150	610	2	1½ day 1* 2½, 2, 2, 2, 2, 2 ,, 2, 3, 4, 5	Neg.
584	June 8 10-15 P.M.	60	604 ...	2½	2, 2 day 2 2, 2, 2, 2 ,, 3, 5	Neg.
88	June 22 2 P.M.	About 100	23 14	2	2, 2 day 2 2, 1½, 2 ,, 3, 4, 5	Neg.
193	1943 Aug. 31 9 A.M.	About 100	265	1	1 day 2 1, 1 ,, 3	Neg.

* 'Day 1' means day on which donor died. On most days two separate exposures of flies were made.

1. Except with goat 610, alternate sides of the receptor's body were used on different days. With goat 610, one side of the body was used after clipping the hair, this being scarified and not cauterized but freshened each day with sand paper.
2. Goat 610 was susceptible to anthrax on retest. The others were not retested, because they died 7-18 days later, from unknown cause or in case of goats 23 and 14 of pneumonia.
3. With goat 265 after exposure to the flies the faecal spots were rubbed into the scarifications with a pestle.

TABLE IV

Musca domestica. Attempted transmission from carcass incisions to eyes of goats.

Number of goat used as donor	Date of death	Number of flies	Duration of Exposure (hr.) on		Interval between the two exposures (min.)	Result	Remarks
			donor	receptor			
88	1942 June 22 2 P.M.	26	½	1	Nil	Neg.	Same receptor used, both eyes being exposed to flies from No. 88 and one eye to those from No. 599.
599	June 29 7-50 A.M.	25	1	½	Nil	Neg.	
113	July 28-29 night.	50	1	1	Nil	Neg.	
78	Sept. 5 ?	50	1½	?	Nil	Neg.	Same receptor goat, right eye for flies from goat 180, left eye for flies from goat 258.
180	Sept 28-29 night.	70	1½	1½	Nil	Neg.	
258	Oct. 14 8 A.M.	60	1½	1½	15	Neg.	
308	Oct. 25 2 P.M.	60	1	1	15	Neg.	Two separate batches of 30 flies fed on same donor and then on two different receptor goats.
192	1943 July 13	30, 30	¾, ¾	½, ½	Nil	Neg.	

Receptor goat No. 78 was susceptible to anthrax on retest, receptor to No. 180 was not tested, while the receptors to Nos. 88 and 113 died from causes other than anthrax.

TABLE V

Bacteriological examination of flies

Number of goat used as donor	Date of death	Number of flies examined	Interval between feeding and killing of flies	Result of cultural examination		Remarks
				Mouth-parts	Bodies	
<i>Stomoxys calcitrans</i>						
152 (carcase)	1941 Aug. 1-2 night	3	Nil	—	..	
165	Aug. 6 ?	50	42 hr.	—	..	Suspension of ground-up flies injected g.-pig. Result negative
154	Aug. 7 1-45 P.M.	3	Nil	—	+	
203	Aug. 14-15 night	2 2	20 min. 42 hr.	? —	+	
203		100	42 hr.	—	—	The flies were killed 19 hr. after third feed on receptor to goat 203 (see Table I). Suspension of flies injected g.-pig: negative
64	Aug. 27-28 night	2 6	22 hr. 44 hr.	— —	+	
157	Sept. 12 2 P.M.	6 5 5 6	Nil 26 hr. 48 hr. 72 hr.	—C —C —G —	+	
215	Oct. 4-5 night	6 6 6 6	24 hr. 48 hr. 72 hr. 98 hr.	— — — —	+	
241	Oct. 18-19 night.	12	Nil	—	+H	
142	Oct. 29 3 P.M.	13 5 5 5 5 5 5	Nil 24 hr. 50 hr. 72 hr. 96 hr. 119 hr. 147 hr.	— — — — — — —	— — — — — — —	Weather becoming cold, flies sluggish and did not feed properly
543	1942 May 1-2 night.	10 20 23	30 min. 18 hr. 42 hr.	— — —	+H +H +C	
538 (carcase)	May 7 7 A.M.	25 39	Nil Nil	—C —C	— —	
31	May 12 10-50 A.M.	9	Nil	+(very few bacilli)	..	Flies fed singly
31		10 10	24 hr. 48 hr.	+H —	
31		27	72 hr.	..	—	Only 5 of the 27 flies alive at time of examination
537	May 15 7 A.M.	20 20 20	Nil 10 min. 30 min.	— — —	— — —	

TABLE V—contd.

Number of goat used as donor	Date of death	Number of flies examined	Interval between feeding and killing of flies	Result of cultural examination		Remarks
				Mouth-parts	Bodies	
591	May 20 9 A.M.	7	Nil	—	+	} Flies fed individually on donor
		7	10 min.	—	+	
		7	30 min.	—C	+	
591		30	24 hr.	..	+	
		30	48 hr.	..	+	
		25	72 hr.	..	—C	25 flies examined after 96 hr. included 17 dead in cage
		25	96 hr.	..	—C	
581	June 1 2 P.M.	8	Nil	+(very few)	+	} Flies fed singly
		7	10 min.	—	+	
		7	30 min.	—	+	
581		25	24 hr.	..	—C	
		22	48 hr.	..	+	
		55	72 hr.	..	+	} Flies fed singly
		6	96 hr.	..	—	
584	June 8 10-15 P.M.	7	Nil	—	+	
		6	10 min.	—	+	
		6	30 min.	—	+	
584		25	40 hr.	..	+	} Flies fed singly
		17	63 hr.	..	+	
		18	87 hr.	..	—C	
28 (carcase)	June 15 6-15 A.M.	100	Nil	..	+	
85 (carcase)	June 18-19 night	100	1 hr.	..	+	
						15 min. after their feed on clipped area on carcass the flies were fed 45 mins. on receptor goat No. 85 (Table II)
88	June 22 2 P.M.	8	20 hr.	..	—C	Flies dead
		25	44 hr.	..	—	Flies dead between 21 and 44 hr. after feeding (Table VI).
		28	68 hr.	..	—C	Flies dead between 46 and 68 hr. after feeding (Table VI)
		26	92 hr.	..	—	Flies dead between 69 and 92 hr. after feeding (Table VI)
88		11	Nil	—	+	Flies fed singly
88		11	20 hr.	..	—	Flies dead
599 (carcase)	June 29 7-50 A.M.	26	5 min.	—	+H	Flies fed on clipped area on carcass
<i>Musca domestica</i>						
85 (carcase)		58	Nil	—	+	A second batch of 21 flies
88 (carcase)		21	30 min.	+	+	
5	July 23	21	30 min.	—	—C	
599 (carcase)		27	Nil	—	+H	
80 (carcase)	Aug. 6 8-40 A.M.	40	2 hr.	..	+	
<i>Calliphora erythrocephala</i>						
610 (carcase)	Aug. 6-7 night	40	2 hr.	..	+	Those 40 flies had been put on cauterized skin of receptor to goat 80 for 1 hr. before being killed (Table II)
						Ditto.

H—Saline suspension of bodies heated 75°C. 30 mins.

C—Badly contaminated plates.

BACTERIOLOGICAL EXAMINATIONS

1.—Examination of mouth-parts and bodies of flies

The specimens were killed with chloroform and their wings pulled off. They were then decapitated when necessary and kept in small Petri dishes or in other glass containers. In a few cases, they were kept overnight in a refrigerator and examined the following day (Table V).

II—Examination of faeces of infected *Stomoxys calcitrans*

The flies, after being fed on infected goats, were kept confined in cages and provided with Petri dishes for deposition of faeces. The dishes were removed from the cage at various intervals for the examination of faeces (Table VI).

TABLE VI
Bacteriological examination of flies faeces

Number of goat used as donor	Date of death	Number of flies	Duration of exposure of donor (hr.)	Period during which faeces deposited after feed on donor	Result	Remarks
31	1942— May 12 . . 10-50 A.M. .	50-60	2	First 24 hr. Second 24 hr. Third 24 hr.	— — —	30-40 flies only alive 20 flies only alive
591	May 20 . . 9 A.M. .	200	1½	First 24 hr. Second 24 hr. Third 24 hr. Fourth 24 hr.	— + (a few) (S) + (many) (S) —	Faeces deposited by about 150 flies Faeces deposited by about 120 flies Faeces deposited by about 25 flies
581	June 1 . . 2 P.M. .	108	2	First 24 hr. Second 24 hr. Third 24 hr. Fourth 24 hr.	— + + —	25 flies dead 22 more flies dead 55 do. 6 do.
584	June 8 . . 10-15 P.M. .	65	?	15 hr. 16-40 hr. 41-63 hr. 64-87 hr.	— — + (few) —	5 flies dead. 25 more flies dead 17 do. 18 do.
88	June 22 . . 2 P.M. .	100	2	20 hr. 21-44 hr. 48-68 hr.	— + —	11 flies dead 25 more flies dead 28 do.

C—badly contaminated plate.

S—anthrax spores in faeces.

SUMMARY

1. Under experimental conditions, *Stomoxys calcitrans* failed to transmit anthrax to goats by its bites or by defaecating on the scarified or cauterized skin of goats.

2. Both *Musca domestica* and *Calliphora erythrocephala* transmitted the disease when brought in contact with the cauterized skin of goats, after having fed on incisions on carcasses of goats dead of anthrax.

3. The disease could not be reproduced by bringing infected *M. domestica* in contact with the eyes of healthy goats.

4. No evidence was obtained of the presence of anthrax bacilli in the mouth-parts of *S. calcitrans*

flies killed at various intervals after their infective feed, except in two instances, in which very small numbers of bacilli were demonstrated in the mouth-parts of flies killed immediately, or within a few minutes after feeding. On the other hand, in some cases the bacilli were found in the bodies of such flies up to 72 hours.

5. Anthrax organisms were found in the faeces of *S. calcitrans* not earlier than 21 and not later than 72 hours after their infective feed.

ACKNOWLEDGEMENTS

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IMMUNIZATION AGAINST RINDERPEST BY A SCARIFICATION METHOD

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(With plate XIII and two text-figures)

AFTER the classical findings of Edwards [1930] in the application of the goat-adapted virus for immunization against rinderpest by the subcutaneous route, there could be little justification for a new approach to the problem, although it is realized, that in practice, under conditions peculiar to India, it had certain limitations of a mechanical nature. The drawbacks lay in the fact that the method needs a number of fragile articles for the operation, and that the vaccine-suspension has to be prepared on the spot and retained for fairly long periods under unfavourable field-conditions.

The disadvantages are more keenly felt in those parts of the country where means of transport are difficult and where public opposition to all forms of cattle inoculation is acute. Obviously, in

these regions it becomes a matter of considerable difficulty to conduct the operation effectively.

Inspiration for an attempt to determine if a suitably modified cutaneous mode of inoculation could be adopted with any possible advantage over the subcutaneous method was drawn from recent advances made in studies of what is known as 'skin' or 'local' immunization.

LITERATURE

Jenner [1800] (cited by Burnet), in his memorable work on vaccination against small-pox, employed the method of local skin infection—the resulting reaction, though mainly localized to the site of inoculation, gave rise to a general immunity. During recent years, the view that the skin is an important immunological organ, as well as serving as a mechanical covering and protecting the vital organs against bacterial invasion, has been supported by many workers.

Besredka [1920] developed 'cuti-vaccination' in certain infectious diseases. He proved that animals could be successfully immunized against anthrax by the intra-cutaneous route and that the immunity thus conferred was of a more solid

With encouragement from Mr T. J. Egan, Director of Veterinary Services, United Provinces, to whom acknowledgements are due, an investigation into the scarification method of immunization against rinderpest was originally carried out by the author in 1938. The work was followed up by other members of the department, and may later form the subject of a separate paper. The scope of this paper is confined to an experiment undertaken at this Institute at the instance of the officer mentioned above.

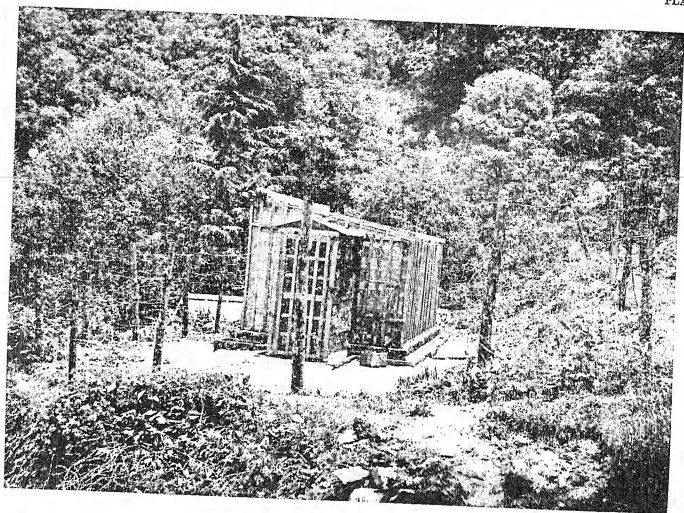


FIG. 1. Fly-proof shed. View from one end.

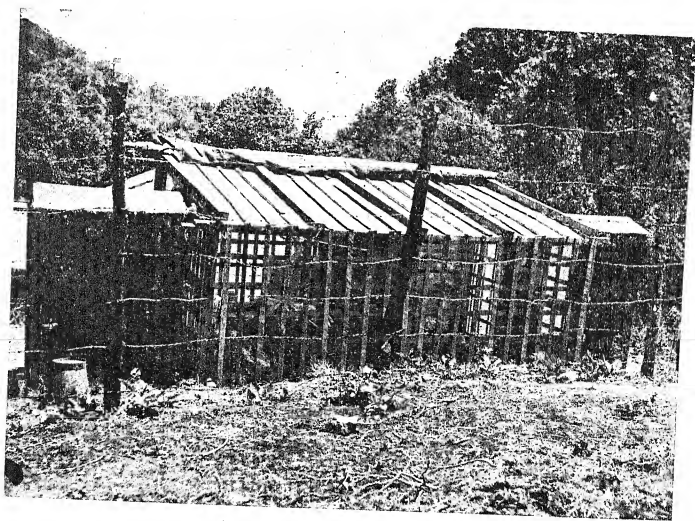


FIG. 2. Fly-proof shed. View from side, showing the two compartments.

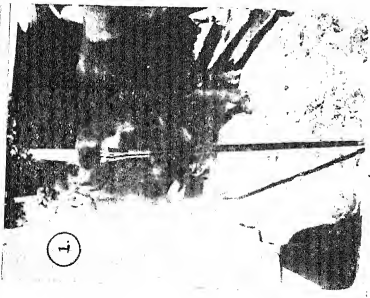


FIG. 1. Sacrificer outfit stick—its use saves an attendant

FIG. 2. Vaccine-pulp being pressed out from the tube into the blade of the sacrificer

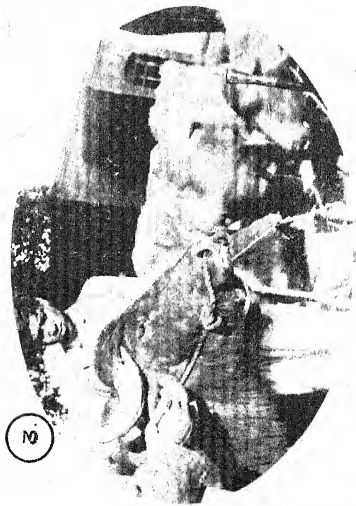


FIG. 3. The operation being performed

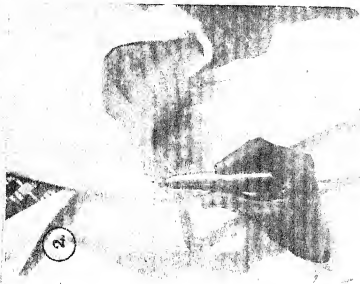


FIG. 4. Appearance of the droplets of serum exudate at the site soon after the operation

nature than that obtained by other methods of inoculation.

Tuft [1931] studied the relative values of various forms of inoculation, with particular reference to the intracutaneous route in immunization against typhoid, and found that the rate of antibody production by the intracutaneous route was equal to that by the subcutaneous route and slightly better than by the intramuscular route, although the total dose of antigen inoculated by the former was one-seventh of the amount introduced by the last two methods. It was also contended that since the skin is richly supplied with reticulo-endothelial cells it may well be adapted for the liberation of the anti-bodies by the stimulus caused at the site of the intracutaneous inoculation.

The experiments of Neufeld and Meyer [cited by Tuft 1931], and a number of other workers have revealed that the reticulo-endothelial system plays an important part in the process of immunity. Tuft [1931] also refers to the possibility of utilizing the skin in a specific manner for the introduction of antigens by other modified forms of inoculation. These consist of injections described as 'percutaneous method' by Petrushky *et al.* through scarification and subsequent inoculation described as 'cutaneous method' by Pondrof, the 'intracutaneous or sub-epidermal method' of Wolff Eisner and Sahli, and the 'anti-vaccination' of Besredka, [all references cited by Tuft (1931)].

These reports suggested the possibility of utilizing the skin for immunization against other infections.

MATERIAL AND EXPERIMENTAL METHODS

The existing subcutaneous method of immunizing against rinderpest was compared with the scarification method in an experimental group of animals. The latter method consisted in bringing about an interaction between the host and the virus through multiple foci of irritation within a small area on the skin of the ear, and was a modification of the intracutaneous mode of inoculation, in that it enabled a relatively larger surface and the entire thickness of the skin to participate in the process of immunization. In this method, the implantation of the virus and scarification of the skin were accomplished simultaneously and thus required no subsequent inoculation. The ear for this purpose offered certain advantages over other parts of the body. It was easier to manipulate, and the site where the antigen had to be implanted, possessed a degree of natural protection against external influences. Being devoid of flesh and provided with thin tender skin, the ear was a favourable site for the points of the scarifier to penetrate to their full depth into the tissues involved.

Scarifier (Plate XIII, fig. 1). The proper length of the points of the scarifier was important. Observations on some dead cattle revealed that the skin thickness on the inner side of the ear varied from 0.8 to 2 mm. Hence, in determining their length due allowance had to be made for such variations, not only in respect of animals of the same breed, but also in those of other breeds, to ensure that the points of the scarifier could pierce the entire thickness of the skin in all breeds, without being long enough to penetrate beyond the layers of the skin.

Vaccine. Goats that had shown a positive reaction were destroyed on the fifth day after inoculation. The spleen was removed and freed from fat and fascia. The organ was then either squeezed in an iron press or ground in a mortar, distilled water being added to the pulp at the rate of 1 c.c. to each gramme, and then transferred to 1 c.c. collapsible tubes—each tube containing about 50 doses. The latter were then wrapped in small pieces of paraffined cloth for storage.

Technique of inoculation (Plate XII, figs. 2 and 3). A drop of vaccine from the tube was allowed to flow and settle uniformly on the blade of the scarifier. A small area devoid of hair and superficial veins on the inner surface of the ear was selected, preferably on the upper border of the lower third region, and the part cleansed without applying any antiseptics. This part was exposed to view and a flat piece of wood held underneath to give resistance. The scarifier was then pressed firmly with a revolving action until contact between the points of the scarifier and the cartilage of the ear could be felt. In most cases this was found quite adequate for depositing the virus on the scarified site, but where it was not satisfactorily lodged it was spread over from the surrounding area with the flat end of the instrument. As soon as pressure on the scarifier was relaxed, droplets of serous exudate could be seen oozing out (Plate XIII, fig. 4) from the lesions and mixing freely with the implanted vaccine. The mixture gradually spread over the whole scarified area and ultimately dried in crusts.

For practical purposes the above process was an indication of a positive 'take', though not with the degree of certainty as in other routes of inoculation. Judging from the brief period during which mixing of the vaccine and skin-exudate occurred, any artificial protection to the area was considered unnecessary.

Animals. Altogether 29 animals, consisting of 25 dairy calves and four hill bulls, were used in the experiment. The calves were 7 to 17 months of age and were born of Hariana animals, immunized to rinderpest and belonging to the Institute herd

The calves were weaned at birth, and fed on milk obtained from immune dairy cows. Of these, 20 calves and the four hill bulls were inoculated by scarification, and the remaining five by the subcutaneous route, with one and the same brew of vaccine.

TABLE I

Reaction following immunization and results of the immunity tests

Animal	Immunized on 7-4-1941			Immunity tests			
	Method of immunization	Reaction	Remarks	Date	Reaction	Result	Remarks
B.C. *	Scarification	No reaction	..	1-8-41	No reaction	Immune	..
Do. *	Do. *	Do. *	..	1-8-41	Do. *	Do. *	..
H.C. *	Do. *	Do. *	..	1-8-41	Do. *	Do. *	..
Do. *	Do. *	Do. *	..	1-8-41	Fever	Partially immune	Recovered
Do. *	Subcutaneous	Fever	..	1-8-41	Mild fever	Do. *	Do. *
Do. *	Scarification	No reaction	..	7-10-41	No reaction	Immune	..
Do. *	Do. *	Mild fever	..	7-10-41	Do. *	Do. *	..
B.C. *	Do. *	Fever 13th and 14th day, partial loss of appetite	B.S.—Neg.	7-10-41	Do. *	Do. *	..
Do. *	Do. *	No reaction	..	7-10-41	Mild fever	Partially immune	Recovered
H. C. 79	Do. *	Do. *	..	7-10-41	H. fever, diarrhoea, loss of appetite	Susceptible	Do. *
B.C. *	Subcutaneous	Fever, partial loss of appetite	..	7-10-41	No reaction	Immune	..
Do. *	Scarification	No reaction	..	12-1-42	Do. *	Do. *	..
Do. *	Do. *	Do. *	..	12-1-42	Do. *	Do. *	..
Do. *	Do. *	Do. *	..	12-1-42	Do. *	Do. *	..
Do. *	Do. *	Do. *	..	12-1-42	Mild fever	Partially immune	Recovered
H.C. *	Do. *	Do. *	..	12-1-42	No reaction	Immune	..
Do. *	Subcutaneous	Mild fever	..	12-1-42	Do. *	Do. *	..
B.C. *	Scarification	No reaction	..	22-4-42	Do. *	Do. *	..
H.C. *	Do. *	Do. *	..	22-4-42	Do. *	Do. *	..
Do. *	Do. *	Do. *	..	22-4-42	Do. *	Do. *	..
H.C. 102	Do. *	Do. *	..	22-4-42	Fever 10th day	Do. *	B.S.—R. Dig
H.B. *	Do. *	H. fever, diarrhoea, loss of appetite	..	22-4-42	No reaction	Do. *	..
H.C. *	Subcutaneous	Mild fever	..	22-4-42	Do. *	Do. *	..
H.C. 104	Do. *	Fever	..	22-4-42	Fever 10th day	Do. *	B.S.—B. Dig.
H.C. *	Scarification	No reaction	Died from causes other than rinderpest on 29-7-41				
B.C. *	Do. *	Fever, partial loss of appetite	Died from causes other than rinderpest on 6-5-41				
H.B. *	Do. *	Delayed fever, diarrhoea, loss of appetite	Died of rinderpest on 20-4-41				
H.B. 152	Do. *	Fever 3rd day only	Died on 29-4-41				
H.B. 416	Do. *	Mild fever	Died on 20-4-41				

B.S. = blood smear H.B. = hill bull B. Dig. = *Bubala dysenteriae* Neg. = negative H.C. = Trifer calf B.C. = bull calf
H. fever = High fever

Reactions (Table I). Of the 20 calves inoculated by scarification, 17 manifested no reaction, local or general, during the two weeks period of observation, while the remaining three calves

showed only a mild thermal reaction. The reactions of these 20 calves are consolidated in a composite chart (Fig. 1). The five calves inoculated subcutaneously showed a moderate thermal reaction

As the number of calves inoculated subcutaneously was less than the number treated by scarification for comparative purposes data were taken at random from an old record of subcutaneously-vaccinated animals* of suitable age and breed, and reactions consolidated into another composite chart (Fig. 2). An examination of these two composite charts will enable a judgement to be formed of the comparative susceptibility of this breed to the two forms of inoculation.

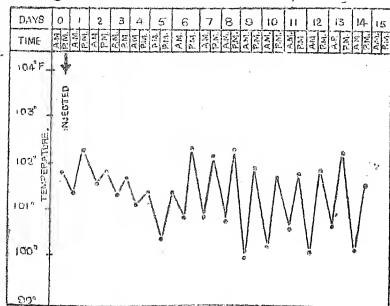


FIG. 1. Composite thermal reaction of the 20 calves immunized by the scarification method under the experiment.

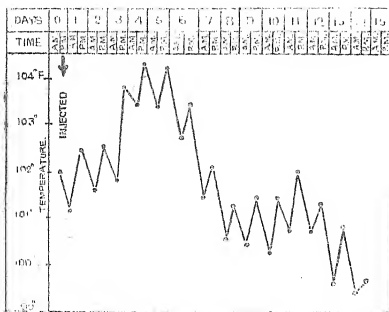


FIG. 2. Composite thermal reaction of the 20 calves immunized by the subcutaneous method in 1937.

*These animals were from amongst a batch of animals immunized as a routine practice with the goat-spleen-virus subcutaneously in 1937, with one and the same brew of vaccine, and in respect of age, breed and immunization history of parents were identical with the animals treated by scarification in our experiment.

Of the four hill bulls, one reacted and recovered a second reacted and died, while the remaining two (Nos. 152 and 416) behaved irregularly, dying without a characteristic reaction on the 22nd and 23rd day. As no hill bulls were inoculated subcutaneously, it was not possible to make any comparison of methods, but in a typical syndrome, this is not what one would expect to find in this highly susceptible breed.

It was also observed that the thermal response in the animals reacting to the scarification route was delayed, commencing on the fifth or the sixth day after inoculation.

Immunity tests (Table I). The animals of the scarification and subcutaneous groups were divided into four similar lots, for immunity test every three months. Two healthy hill bulls were also included to serve as virus controls at each test. All were injected subcutaneously with 5 c.c. of virulent hill bull blood, and in addition, the animals under test were allowed to mix freely with the reacting controls, so that they were exposed to all possible outside chances of infection.

Of the scarification group consisting of 19 animals which remained for test there was evidence of strong immunity in 15 (including the remaining hill bull), partial immunity in three and in one (H. C. 79) apparently no protection. Among the subcutaneous lot of five calves, immunity was strong in four, and partial in one. One calf (H. C. 102) of the scarification group and one (H. C. 104) of the subcutaneous group, though immune to rinderpest, showed resuscitation of blood protozoa during the test. In no case, however, was it necessary, either during immunization or test, to control the reaction by serum. The eight hill bulls serving as test-virus controls reacted typically and died.

DISCUSSION

It is evident that in most of the calves there were no reactions following immunization by scarification. In a few, however, there was a mild thermal response. It is also evident, that in spite of the absence or mildness of the reactions, the immunity conferred by this method could be favourably compared with that obtained by the subcutaneous method. Our results in respect of reactions are apparently in agreement with those of Tuft [1931] in the case of a potent mixed typhoid vaccine. This worker immunized over 100 individuals with small doses of this vaccine intracutaneously, obtaining results which were usually better than those got with larger doses by other routes and without general reactions. Absence of reaction or its delayed appearance and mildness may be attributed to the smallness of the dose of inoculum absorbed, to its local fixation, or to slow rate of its absorption. From a practical

standpoint, absence of recognizable local or general reaction during immunization may be regarded in one sense as a disadvantage. To prove that absence of reaction in vaccinated animals does not necessarily indicate failure to induce immunity, it would be necessary to vaccinate and test a considerable number of animals. (Further experiments on an extensive scale are in progress). Some attempt towards this end in the case of rinderpest is provided by the experiments recorded in this paper.

The results in the case of hill bullocks indicate that by the present technique the method cannot be used safely in this breed, owing to their very high susceptibility.

Hall [1934] found that the minimal amount of whole blood that could set up a (delayed) reaction in animals susceptible to rinderpest was 0.0002 c.c. It would be impossible to measure the amount of antigen absorbed from the scarified site, but considering the concentrated nature of the vaccine-pulp implanted and the minuteness of the dose of virus required to infect an animal, it may be presumed that the amount absorbed would exceed this limit.

Some Russian authors [cited by Hutyrá, Marek and Manninger, 1938] were inclined to believe that the natural infection occurred as two distinct types, viz., exanthematous and non-exanthematous. Should this difference of virus type be established, it may be possible to adapt the exanthematous strain to growth in dermis. In this case the use of such a virus, either alone or with the addition of a suitable tissue depressant, might cause the antigen to be fixed, resulting in local pustule formation and so rendering the host immune to both virus types.

It may be admitted that infective agents like those of anthrax and small-pox, where vaccination through intracutaneous route has given encouraging results, may have had a special predilection for the skin. On the other hand, with infections like typhoid which are not known to possess any marked predilection, equally favourable results were obtained by Tuft [1891]. This finding in the case of rinderpest, which some believe to have a predilection for the epithelial tissues and also in a proportion of cases for the skin, should apply with a greater degree of certainty where the antigen employed is a living virus capable of proliferation. It may also be added, as a fact of common knowledge, that local irritation of skin sites favours the proliferation of virus implanted thereon.

The simplicity of the operation, the considerably smaller amount of apparatus and attendants

needed, the ease with which the method can be adopted, particularly during expeditions and war conditions, and the possibility of its being more readily acceptable to Indian stockowners, owing to its close resemblance to small-pox vaccination, with which they have been familiar for decades, would appear to be facts of importance.

SUMMARY

The possibility and manner of applying goat-adapted rinderpest virus by scarification is reported. Results obtained by the two routes of inoculation, viz., scarification and subcutaneous, are compared. The simplicity of the operation and other advantages accruing to the scarification method are pointed out.

The chief points brought out by the experiment are:

1. The ease of performing the scarification method and the smaller amount of apparatus and attendants required are considered to be its chief advantage over the subcutaneous method.

2. Results indicate that in most cases reactions following immunization by scarification are slight or absent.

3. Out of 19 animals tested at intervals up to a year, immunity was strong in 15, partial in three, and apparently absent in one. Of the five animals immunized subcutaneously, immunity was strong in four and partial in one.

4. Absence of reaction in the scarification method appeared to be without effect on the resulting immunity.

5. The scarification method by its present technique cannot be safely employed in hill cattle of Kumauti breed.

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The author is grateful to Dr F. C. Minett, to Mr J. R. Haddow, and to Mr S. R. Hassan, for helpful suggestions and facilities, and to the first-named for assistance in writing the paper.

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POSSIBILITIES OF ADDING SESAME OIL TO HYDROGENATED FAT OR VANASPATI, TO DISTINGUISH IT FROM GHEE

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(Received for publication on 14 August, 1943)

SEVERAL varieties of hydrogenated vegetable fats have been put on the market at present, which closely resemble ghee in almost all their physical characteristics. To distinguish ghee from such hydrogenated fats has therefore become a matter of great concern with the consumer. The problem is analogous to the problem of butter *vs.* margarine in Western countries. To facilitate the detection of adulteration of butter with margarine, it has been made compulsory in Austria, Belgium, Denmark, Finland, Germany, Portugal, Sweden and Switzerland to add 5 to 10 per cent of sesame oil to the latter. By this means margarine is easily distinguished from butter by the well-known Boudanin or Villavecchia test. This investigation was therefore undertaken to explore the possibilities of adding sesame oil to hydrogenated fats to facilitate their detection when mixed with ghee.

In the past whenever this subject received attention from the authorities concerned, objections were raised to the adoption of such a precautionary measure by the manufacturers of hydrogenated fats on the ground that, as sesame oil had a relatively high iodine value, it was likely to lower their keeping quality. No data had,

however, been obtained so far to prove if this was really so and to what extent the quality of hydrogenated fats was actually likely to be affected by the addition of sesame oil in different proportions. This and related aspects of the problem have therefore been carefully determined by this investigation.

EXPERIMENTAL

(1) *Effect of adding sesame oil on the keeping quality of hydrogenated fats.* Two different commercial brands of hydrogenated fats, one made from groundnut oil (Brand A) and the other from cottonseed oil (Brand B) were used for these studies. Sesame oil, after being freed from any free acids and suspended matter, was mixed with these samples in the proportions of 5, 10, 15 and 20 per cent. The samples were kept in glass bottles and exposed to diffused light. A sample of each of the two original brands was also kept under identical conditions as control. The keeping quality was measured in terms of peroxide value at intervals by the method described by Dastur and Lea [1940]. The results obtained are given in Tables I and II. The peroxide values are expressed in terms of N/500 sodium thiosulphate.

TABLE I
Peroxide values of hydrogenated fat Brand A (ml. of N/500 thiosulphate)

Weeks	Original	IV	VIII	XII	XVII	XX	XXIV	XXVIII
Hydrogenated fat	1.33	3.80	9.93	14.33	14.92	19.95	25.39	31.37
5 per cent S. oil	1.69	4.86	11.87	16.38	17.71	22.92	28.34	35.08
10 " " "	2.72	5.14	13.04	18.04	19.98	24.83	30.80	37.69
15 " " "	2.86	5.86	14.80	19.77	22.32	28.36	35.27	41.87
20 " " "	2.94	6.44	16.60	22.82	25.64	31.53	39.76	47.82

TABLE II
Peroxide values of hydrogenated fat Brand B (ml. of N/500 thiosulphate)

Weeks	Original	IV	VIII	XII	XVII	XX	XXIV	XXVIII
Hydrogenated fat	1.66	2.75	5.89	8.14	8.94	13.67	17.87	22.22
5 per cent S. oil	2.30	3.77	9.12	12.25	13.98	18.26	22.39	26.90
10 " " "	3.25	4.93	11.46	16.32	15.00	21.18	26.34	31.50
15 " " "	3.33	5.63	13.09	18.03	22.69	27.13	32.69	37.83
20 " " "	3.65	6.13	16.49	21.29	25.17	32.28	37.94	44.81

On keeping fats containing sesame oil, the intensity of Villavecchia reaction tended to diminish. In spite of this, it was possible to detect the presence of sesame oil in ghee adulterated with 1 per cent of hydrogenated fats containing 5 per cent sesame oil and stored for seven months. Hence this decrease in colour-giving property of hydrogenated fats containing sesame cannot be a disadvantage.

Butterfat obtained by feeding excessive amounts of sesame cake (average crude fat=14 per cent) did not give the Villavecchia reaction, though there was a considerable alteration in other chemical characteristics of such butterfat. Hence a positive test for sesame oil in ghee can safely be relied upon to indicate the presence of hydrogenated fats containing sesame oil.

It is very likely that different samples of sesame oil would give varying colour intensity in the Villavecchia reaction. Also, if sesame oil, previous to its being added to vanaspati, is heated to high temperatures (above 225° C.), it may lose the property of giving a positive Villavecchia test. Further, by filtering sesame oil through different absorbing media, like charcoal, Kiesulguhr etc., similar effects may be produced [cf. Gravidorst, 1924]. To overcome these defects, if the idea of adding sesame oil to hydrogenated fats is ever put into practice, the best recourse would be to specify that when ten grams of fat are tested by the stand-

ard Villavecchia test, a colour intensity of say at least 15 red units should be produced.

This would require an addition of only 5.7 per cent of sesame oil to hydrogenated fats; and under these circumstances, as shown before, the keeping quality, flavour, etc. of these products would hardly be affected. Addition of 1 per cent of such fats to ghee can be detected without any difficulty with improved Villavecchia reaction [Dastur, *et al.*, 1943] and hence adulteration of ghee with this medium would hardly be worth attempting.

SUMMARY

1. Addition of 5-10 per cent of sesame oil to hydrogenated fats does not have any appreciable adverse effect on the keeping quality of the fat.

2. Addition of 1 per cent of hydrogenated fats, containing sesame oil in such amounts, to ghee is easily detected with the help of improved Villavecchia reaction.

3. Butterfat secreted by cows and buffaloes fed excessive amounts of sesame cake does not give the characteristic colour reaction for sesame oil.

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SOME OBSERVATIONS ON *AMOEBOTAENIA SPHENOIDES* FROM POULTRY

By S. H. QURASHI, Assistant Disease (Poultry) Investigation Officer, United Provinces

(Received for publication on 13 July, 1943)

AMOEBOTAENIA SPHENOIDES has been reported as a very common parasite of fowls. Southwell [1921, 1930] and Meggitt [1926, 1931] have described this species in detail. Recently, the writer collected a large number of these parasites during *post-mortem* examinations of fowls, and it has been possible to obtain quite a fair number of complete worms. The observations made on these specimens reveal some new facts.

Southwell [1930] mentions that the maximum length of specimens of this species is 2 mm. Bhalerao [1935] mentions the length of this species as 2.4 mm. Usually, the length of the specimens of this species is more than 2 mm., but, as some of the mature segments fall off, the remaining worm shows a smaller length. This is also the case with the smaller immature worms. A greater variation is seen

in the number of segments. While Southwell [1930] mentions that the number of segments varies from 13 to 24, Bhalerao [1935] gives the maximum number as 24. It, therefore, appears that the specimen collected, heretofore, did not show more than 24 proglottides. The collection made by the writer shows that most of the worms have a greater number of segments than 24. In fact, the number varies from 24 to 30.

The account, hitherto given, seems to have been based on the length of incomplete worms.

There is also a good deal of variation in the number of testes, which have been mentioned as 12 or more. This also does not seem to be a fixed number. In fact, in the specimens, which are longer than 2 mm. and in which the number of segments is also more than 24, the number of testes is about 9-10. The larger

number of testes (12) reported has not been seen even in one single specimen obtained by the writer.

The differences noted above, although fairly important, do not, however, justify the creation of a new species and may prove to be individual variations. In view of these observations, it may be considered advisable for the present to amend the specific diagnosis of *Amoebotaenia sphenoides* as follows:

Specific diagnosis of Amoebotaenia sphenoides
Railliet 1899 emended

Diagnosis. *Amoebotaenia* with a length up to 4 mm.; number of proglottides up to 30. Testes less than 12 or 12, running along the posterior margin of the proglottides. Uterus persistent, lobed posteriorly. Cirrus armed with minute spines.

Host—Fowl.

Location—Intestine.
Locality—United Provinces (Lucknow, Kasganj and Shikohabad).

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TRICHURIS VULPIS IN DOGS IN INDIA

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(Received for publication on 20 March, 1944)

In spite of the fact that parasites belonging to the genus *Trichuris* are so common and widespread in man and ruminants in India, there has been no record of their occurrence in dogs in this country, except in the recent publication of Srivastava [1942]. More during the examination of material from dogs at Mukteswar and elsewhere, he says that he came across half a dozen cases of whipworm infestation. He observes that the infestation is fairly widespread in this country in view of the fact that the material examined by him came from different and distant localities, such as Bombay, Jind State, Bhimtal and Dehra Dun.

Below are given some details of a case observed by the writers.

HISTORY

On 21st April 1943, a three months old cocker spaniel, ordered by an American military officer, was admitted into the Dog Hospital of the Bengal Veterinary College for treatment. According to the history supplied by the owner, the pup was born and reared in Tennessee State of the U. S. A. and was brought to Calcutta at the age of two months, in apparently good health. From about a week after its arrival in Calcutta, the pup remained in Assam. Shortly after its return to Calcutta, it became ill, had low fever with

diarrhoea, occasionally tinged with blood and mucus, resulting in considerable loss of condition and rapid emaciation.

The pup was found on examination to be in a very poor condition. Temperature records revealed only a moderate rise above the normal. The faeces continued to be loose.

Distinct anaemic changes were noticed in blood films. Microscopical examination of the faeces, after floatation with saturated sugar solution, repeatedly revealed worm eggs of two different types in fairly large numbers. They were identified as (i) *Ancylostoma* eggs and (ii) *Trichuris* eggs, the latter predominating.

A course of general tonic was administered and, after the results of the faecal examination were known, a small dose of tetrachlorethylene was also given. This, however, proved of no avail, and the pup died about a fortnight after admission to hospital.

POST-MORTEM FINDINGS

When examined after death the internal organs did not show any significant abnormality beyond the pale and comparatively bloodless condition generally obtaining in anaemic and debilitated animals. From the caecum and partly from the large colon, 143 whipworms and two dead hookworms were collected. Of the 143 whipworms, 61

turned out to be males and the remainder were females. A search for lesions in the mucosa of the caecum did not reveal any well-pronounced or clear-cut lesions, except a few scattered petechiae and at places an ill-defined and superficial erosion, but it was found to be coated with a fair amount of mucus and gave an impression of being somewhat thicker than normal.

DISCUSSION

As stated above, Srivastava considered that *T. vulpis* infestation was widespread in India from the fact that the material examined by him came from about three different and distant localities. The experience of the present writers who have examined several hundreds of faecal specimens from dogs in Calcutta and have conducted post-mortem examination on a large number of dog carcasses, and those of other workers who have from time to time reported on the parasitic fauna of Indian dogs, has been against such an assumption. It may be recalled that the pup reported upon in this paper was an imported dog, and it is evident from the available history that the infestation took place

before the pup arrived in India. Unfortunately, Srivastava does not state whether the dogs were imported or indigenous. It seems very likely that his material also originated from imported dogs. It does seem surprising that *Trichuris vulpis* has not yet become an established parasite of the indigenous dog, in spite of the fact that for many years large numbers of dogs have been imported into the country from different parts of the world, including those places where *Trichuris vulpis* is known to occur.

ACKNOWLEDGEMENT

Thanks are due to Mr D. N. Das, G. V. Sc., House Surgeon, Bengal Veterinary College, for kindly supplying the clinical history.

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[Note.—From literature which became available after the submission of the above note to the press it appears that eggs of *T. vulpis* in dog faeces have been noticed by other workers before Srivastava. Kuppaswamy (An. Rep. V. I. O., Orissa, 1940-41 and 1942-43) found them in three dogs, but in this case also the details concerning the origin of the infested dogs are not available.]

STATISTICAL STUDY OF A BREEDING EXPERIMENT WITH GOATS

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THE experiment was started in 1931-32 with a foundation stock of 36 Barbari does, nine female kids and three bucks. Six more does and one buck were purchased sometime later during the year 1935-37, thus bringing the total number of purchased goats to 51. The three original purchased bucks were given the registered numbers 48M, 49M and 50M, and the fourth buck purchased later was numbered 631M. The number of female kids obtained from the foundation stock from each of these bucks and which completed at least one lactation on the farm is given in Table I. Four more

Some of the foundation stock was also mated to the male progeny of 48M and 49M. The numbers of daughters obtained from these are given in Table II. In all, there were 34 daughters obtained from 19 mothers of the foundation stock. For convenience, we shall hereafter call them the first progeny.

TABLE II

Buckwise distribution of daughters of the first Progeny

Buck No. (1)	Total number of daughters from these (2)	Number of daughters completing at least one lactation (3)
144M . .	5	3
152M . .	5	2
633M . .	2	2
Total . .	12	7
Unknown . .	?	4

TABLE I
Buckwise distribution of daughters of the first progeny

Buck No. (1)	Total number of daughters from these (2)	Number of daughters completing at least one lactation (3)
48M . .	26	14
49M . .	16	8
50M . .	3	1
Total . .	45	23

daughters kept on the farm have not been entered in this table as their mothers were bred unnoticed.

Out of the daughters born to the first progeny from stud-bucks on the farm 29 completed one or more lactations. These 29 are called the second progeny. The buckwise origin of this progeny is given in Table III. The third progeny which came in milk before 31 March 1941 numbered

10 only. Their sires are given in Table IV. There are also two daughters of the fourth progeny which came in milk before March 1941 but their sires are unknown and no use is made of their records in this report.

TABLE III

Buckwise distribution of daughters of the second progeny

Buck No. (1)	Total number of daughters from these (2)	Number of daughters completing at least one lactation (3)
48M . .	4	2
49M . .	9	2
50M . .	7	4
144M . .	10	8
152M . .	25	1
631M . .	1	6
633M . .	15	1
Total . .	71	24
Unknown . .	?	5

TABLE IV

Buckwise distribution of daughters of the third progeny

Buck No. (1)	Total number of daughters from these (2)	Number of daughters completing at least one lactation (3)
333M . .	8	1
389M . .	15	1
631M . .	2	2
633M . .	13	5
Total . .	38	9
Unknown . .	?	1

The farm-bred bucks used on the farm have the following pedigrees. Pedigrees of the original bucks are not known.

152 M	{ 48M Purchased Dam 53
333M	{ 48M Purchased Dam 82
389M	{ 152M 1st Progeny Dam 297
633M	{ 144M 1st Progeny Dam 221
144M	{ 49M Purchased Dam 74
	{ 48M Purchased Dam 66

The performances of the dams involved in these pedigrees are given in Table V. A few other bucks were also used on the farm during this period but their daughters either died or were not in milk till March 1941.

TABLE V

Performances of dams involved in the pedigrees of stud-bucks

Date of kidding	milk yield during lactation oz.	Average milk yield during lactation oz.	Number of days in milk	Average yield during kidding interval oz.
<i>Dam No. 53 of the foundation stock</i>				
6-12-31	4363	17	255	14.4
4-10-32	5633	19	296	13.8
22-11-33	5376	25	217	14.8
21-11-34	4356	33	133	23.0
29- 5-35	1344	24	56	...
Average	4234.4	22.1	191.4	15.6
<i>Dam No. 74 of the foundation stock</i>				
18-12-31	7158	32	226	14.1
7- 5-33	4294	20	213	13.4
24- 3-34	3142	26	123	9.3
27- 2-35	2884	41	70	...
Average	4369.5	27.7	158.0	12.3
<i>Dam No. 82 of the foundation stock</i>				
17-11-31	3918	13	309	7.4
1- 5-33	5044	25	205	18.8
25- 1-34	5530	28	195	18.1
27-11-34	4720	28	169	13.6
10-11-35	1776	16	110	...
Average	4197.6	21.2	197.6	14.5
<i>Dam No. 66 of the foundation stock</i>				
20-3-32	7086	30	235	23.2
19-1-33	5440	29	189	...
Average	6263.0	29.5	212.0	23.2
<i>Dam No. 221 of the first progeny</i>				
17-10-34	5804	36	162	28.7
7- 5-35	4550	36	127	25.0
5-11-35	7560	37	204	20.5
8-11-36	6664	36	185	35.8
13- 5-37	4600	39	118	25.7
8-11-37	6906	44	156	32.9
6- 6-38	2390	33	72	...
Average	5496.3	37.6	146.3	28.1
<i>Dam No. 297 of the first progeny</i>				
10- 1-36	7628	35	219	19.3
18- 2-37	5560	46	188	30.5
26-11-37	Remained sick during lactation period			
5-12-38	7484	37	205	26.6
12- 9-39	5609	35	169	23.4
9- 5-40	2374	24	97	12.7
20- 2-41	4684	32	146	20.2
Average	6056.5	35.5	170.7	22.1

TABLE VI

Comparison between the performance of the foundation stock and their first progeny

		Number of goats	Number of lactations	Total milk yield oz. (4)	Length of lactation days (5)	Average milk yield per day of lactation oz. (6)	Number of goats	Number of lactation	Length of kidding interval days (9)	Average milk yield per day of kidding interval oz. (10)
(1)		(2)	(3)				(7)	(8)		
Foundation stock	Average	19	68	4751.8	176.7	26.9	16	48	312.5	16.2
	Standard error			198.5	8.4	1.8			16.9	1.5
First progeny	Average	34	130	5832.5	176.1	33.1	30	110	291.4	20.9
	Standard error			210.3	5.2	1.4			9.5	0.8
<i>t</i>				3.3*	0.1	2.9**			1.1	3.5**

The object of this note is to analyse the records maintained on the Farm with a view to find out the extent to which the breeding policy followed on the Farm during the last 10 years has resulted in the improvement in the milk yield of the herd. The appropriate method to assess the improvement would be to compare in successive progenies, the total milk yield during lactation in relation to the length of lactation and the length of kidding interval. In other words, we have to compare the performance for total milk yield by standardizing, as it were, the length of lactation and kidding interval. This is done by comparing the values for (1) average milk yield per day of lactation and (2) average milk yield per day of kidding interval.

Table VI gives the set of comparisons between the performances of the foundation stock and the first progeny. Columns (2) and (7) of the table show the number of goats in the two generations whose records have been compared. Columns (3) and (8) give the number of lactations to which the average performances shown in the other columns refer. Columns (4), (5), (6), (9) and (10) give the average values with their standard errors of the total yield during lactation, the length of lactation in days, the average daily milk yield during lactation, the kidding interval, and the average daily milk yield during the kidding interval respectively. It will be seen that while there is a slight decrease in the length of lactation (from 176.7 to 176.1 days) and the kidding interval (from 312.5 to 291.4 days) the first progeny has given a higher total milk yield as also a higher average daily milk yield during the lactation and the kidding interval. But this comparison without reference to the errors of the averages compared is by itself inadequate to support the conclusion regarding the superiority of the first progeny over their mothers. For the averages which have been compared are themselves variable in the sense that different values for the performances would have been obtained had there been different numbers of goats in the two generations. Before we conclude that the first progeny is superior to their mothers, it is therefore necessary

to satisfy ourselves that the difference observed between the performances of the two generations is larger than the possible difference arising from variability of the averages. This variability of the averages is measured by what is called the standard error and the statistical method to judge whether the difference observed is due to chance causes is known as the *t* test of significance. When the difference observed cannot be explained on the grounds of chance, i.e., it is larger than can be expected on chance causes alone, the difference is said to be statistically significant. The values for the standard error have been shown below the respective averages and the values of *t* are shown in the last line of the table. Whenever the observed difference is found to be significant, the fact is indicated by asterisks against the corresponding values of *t*.

It will be seen from the table that there is no significant difference between the length of lactation and kidding interval of the two generations but that the first progeny has given a statistically superior performance over their dams in respect of all the other three characters. This may be due to several factors: (1) the sires may have had a real transmitting ability, (2) the better feeding and herd management right from the birth of the first progeny in contrast with the environment in which the dams were reared may also be partly responsible, (3) the existence of hybrid vigour which is frequently observed in the progeny of unrelated animals may also partly offer an explanation, or (4) there may have been a seasonal effect arising from the different climatic and disease conditions in the different years in which the dams and their daughters had their respective lactations. The better feeding and management at the farm is expected to have a beneficial effect on the performance of the first progeny but it is obviously impossible to isolate for study the effect of this factor. We have, however, gone into considerable detail in regard to factors (1) and (4). Table VII shows the sirewise comparison of the first progeny and their mothers.

TABLE VII

Progeny tests for bucks 48M, 49M and 50M

			Number of goats	Number of lactations	Total milk yield oz.	Length of lactation days	Average milk yield per day of lactation oz.	Number of goats	Number of lactations	Length of kidding interval days	Average milk yield per day of kidding interval oz.
			(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
48M	Foundation stock	Average	11	38	4498.1	173.6	25.0	9	24	209.5	17.5
		Standard error			302.5	12.5	2.3			20.8	1.7
	First progeny	Average	14	55	6199.5	178.2	34.8	12	45	286.9	23.2
		Standard error			353.8	8.1	4.4			16.2	0.8
	<i>t</i>				3.4**	0.3	2.8**			0.5	3.2**
49M	Foundation stock	Average	6	25	4392.7	161.4	27.2	5	18	293.4	15.4
		Standard error			269.1	10.3	3.5			20.6	2.4
	First progeny	Average	8	35	4920.7	157.0	31.3	7	31	268.5	18.2
		Standard error			340.6	7.6	8.0			15.2	2.0
	<i>t</i>				1.1	0.3	0.9			1.0	1.0
50M	Foundation stock	Average	1	3	5702.0	197.3	23.9	1	2	302.5	22.5
	First progeny	Average	1	5	5891.2	167.4	35.2	1	4	337.5	17.0
	<i>t</i>										
Values of <i>t</i> for the comparison of the bucks 48M and 49M											
	<i>t</i>				1.8		0.7				0.8

It will be seen that the performance of the first progeny over their dams for sire 48M alone is significant, suggesting that this sire was a good transmitter provided the superior performance of the first progeny was not brought about by better conditions in the later years. The performances of the first progeny sired by bucks 49M and 50M were not significant. The significance of the difference in the climatic conditions can be best appreciated from Table VIII, showing the distribution of lactations of the foundation stock and the first progeny in the different years. Each year has been divided in this table according as the lactations followed the summer or the winter kidding. It will be seen from this table that in the case of foundation stock the majority of the lactations were completed before 1934 summer while in the case of the first progeny the majority of the lactations were completed during the period from 1934-38. It will also be seen that there is relatively a larger number of winter kiddings in the first progeny than in the foundation stock. Hence the difference in the performance of the foundation stock and of the first progeny may be to a certain extent attributed to the difference in the two periods and to the unequal numbers of winter and summer kiddings in the two progenies. As, however, we do not know how and which of the climatic factors influence the milk yield, it is hardly possible to make any allowance for the seasonal effect. The best we can do is to compare the lactations of the two groups falling in common years, although even here the first progeny will have an advantage over their mothers in so far as it will constitute a comparison between the

TABLE VIII
Distribution of lactations

	48M		49M	
	Dams	Daughters	Dams	Daughters
1931 W	5	..	2	..
1932 S	3	..	2	..
W	3	..	1	..
1933 S	5	..	6	..
W	3	..	1	..
1934 S	3	1	4	2
W	3	3	3	3
1935 S	4	3	2	2
W	1	9	3	5
1936 S	..	1	..	5
W	1	5	..	3
1937 S	1	6	2	4
W	2	5	..	5
1938 S	..	1	..	1
W	..	4	..	4
1939 S	..	4	..	1
W	2
1940 S	..	2	..	2
W	1
Total	34	44	26	40

TABLE IX

Common year lactation comparison

			No. of lactations	Total milk yield oz.	Average milk yield per day of lactation oz.	Average milk yield per day of kidding interval oz.
48M	Foundation stock . . .	Average . . .	15	5178.7	34.6	18.2
		Standard error . . .		721.0	2.4	3.0
	First progeny . . .	Average . . .	33	6627.8	36.5	20.9
		Standard error . . .		378.1	2.2	1.5
	<i>t</i>			2.0	1.0	0.9
49M	Foundation stock . . .	Average . . .	12	4450.7	30.9	19.0
		Standard error . . .		596.0	7.4	3.0
	First progeny . . .	Average . . .	12	4670.7	32.1	18.3
		Standard error . . .		530.1	5.3	1.8
	<i>t</i>			0.3	0.5	0.2

earlier lactations of the first progeny and the later ones of their dams. The results of these comparisons are shown in Table IX, for bucks 48M and 49M. Buck 50M does not provide sufficient material for such a comparison. It will be seen that the first progeny of sire 48M gives a significantly larger total milk yield per lactation. When, however, the total milk yield is corrected for the length of lactation and the kidding interval, the difference disappears. The progeny of sire 49M does not show a significant difference in any of these characters. Table IX-A shows the results of the separation of the common year lactation comparison for the progeny of buck 48M into summer and winter kiddings. It will be seen from the table

TABLE IX-A

*Separation of the common year lactation comparison
for the progeny of buck 48M into summer
and winter seasons*

		No. of lactations	Total milk yield oz.	Average milk yield per day of lac- tation oz.	Length of lac- tation days
Summer	Foundation stock . . .	8	3952.0	32.8	120.4
	First progeny . . .	11	5004.8	34.9	143.4
	<i>t</i>		1.14		
Winter	Foundation stock . . .	7	6580.8	36.0	183.0
	First progeny . . .	22	7440.0	37.3	199.0
	<i>t</i>		0.98		

that there is no significant difference between the performance of the first progeny and of their mothers within a season, but all the three characters give a significantly higher value for the winter than for the summer lactation. As the differences are very large, the values of *t* have not been given. The preponderance of winter lactations in the common period in the first progeny observed in Table VIII thus at once partly explains their superiority over the foundation mothers. We may, therefore, conclude that while breeding may have had an effect, the better performance of the first progeny might also to an extent be due to better feeding and management at the farm, to differences in the years in which the majority of the lactations of the foundation stock and the first progeny were completed and to the preponderance of winter kiddings in the first progeny.

While bucks No. 48M and 49M are by themselves not proved it is of some interest to compare their relative worths as transmitters of milk yield. This is done in the last row of Table VII giving the values of *t* for the comparison of the transmitting abilities of the two bucks, transmitting ability being measured by the difference in the performances of the progeny and their mothers. It will be seen that these comparisons are free from consideration of the order of lactation or seasonal effects. The table shows that none of the three values of *t* is significant, though the value of *t* for total milk yield is high and approaching the 5 per cent level of significance. While, therefore, there is a slight suggestion that buck 48M is probably superior to 49M, there is no adequate evidence to establish his superior worth.

We shall now consider the results of the comparison of the second progeny with their mothers in the first progeny. These are given in Table X. Unlike Table VI, where a collective comparison over all lactations has been made owing to lack of information regarding the order of lactations of the foundation stock, the comparison in this table is made for corresponding lactations. It will be

seen that far from showing an improvement the results indicate significant deterioration in all the lactations. The material is too scanty to allow examination of seasonal effect in the way we did in Tables VIII and IX. While season may have had an effect one way or the other, the data leave little doubt in our mind that the bucks had little worth as transmitters of milk yield.

TABLE X

Comparison between the performance of the second progeny and their dams

		Number of goats	Total milk yield oz.	Number of days in milk	Average milk yield per day oz.	Number of goats	Kidding interval	Average milk yield per day of kidding interval oz.
First lactation	1st progeny							
	Average	18	5396.3	167.8	32.2	18	264.3	20.5
	Standard error		423.6	11.9	1.1		23.1	1.2
	2nd progeny							
	Average	27	4864.1	176.9	27.5	25	313.7	14.8
	Standard error		349.7	11.9	0.9		23.7	1.2
	<i>t</i>		0.9	1.7	3.2**		1.4	3.6**
Second lactation	1st progeny							
	Average	15	5558.4	152.6	36.4	11	257.1	23.6
	Standard error		131.4	8.8	1.4		22.5	1.3
	2nd progeny							
	Average	15	5216.0	192.0	27.2	12	0.0	15.7
	Standard error		102.5	9.8	1.4		3328.7	1.5
	<i>t</i>		0.6	3.0**	4.8**		1.9	3.8**
Third lactation	1st progeny							
	Average	7	7906.3	206.0	38.4			
	Standard error		1416.6	28.2	2.1			
	2nd progeny							
	Average	7	5617.4	215.7	26.0		Sufficient data not available	
	Standard error		931.3	29.6	2.2			
	<i>t</i>		1.4	0.2	4.1**			

Table XI shows the results of the progeny tests for bucks Nos. 152M and 633M having more than five daughters. As little reliance can be placed on the progeny tests involving less than five comparisons, these are not made. It will be seen that the progeny of 152M does not show a significantly different performance from that of their mothers but that the progeny of 633M suggests a significant deterioration. The comparison of the relative worth of the two sires, however, made in the last row of the table does not suggest that 633M was a distinctly inferior buck to 152M.

The results of the comparison of the third progeny with their dams in the second progeny are given in Table XII. It will be seen that while there is some increase in the total milk yield, there is a significant reduction in the average milk yield

per day of lactation and a considerable though non-significant reduction in the average milk yield per day of kidding interval. This corroborates to a great extent the findings of Table X.

The comparisons so far made are those between the successive progenies and their mothers, and although appropriate from the view-point of testing the success of breeding policy do not give us a complete picture of the improvement in the performance of the herd as a whole in successive generations. This is done in Tables XIII and XIII B, which give the performance over all lactations of the foundation stock, of the first progeny and lactationwise performance of the first, second and third progenies. It will be seen that while the first progeny shows a better performance over the foundation stock, there is a steady deterioration in the performance thereafter.

TABLE XI

Progeny tests for bucks 152 M and 633 M

		No. of goats	Total milk yield oz.	No. of days in milk	Average milk yield per day oz.	No. of goats	Kidding interval	Average milk yield per day of kid- ding interval oz.
152M	1st progeny	5	5200.8	165.0	31.5	5	299.4	17.4
	Average		991.1	29.7	1.8		63.2	1.4
	Standard error . . .							
	2nd progeny	8	4560.0	155.8	29.3	7	268.3	15.2
	Average		541.0	15.0	0.7		36.5	1.2
	Standard error . . .							
			0.6	0.3	1.1		0.4	1.1
633M	1st progeny	5	5290.0	174.8	30.3	5	271.4	19.5
	Average		890.4	27.1	0.9		34.1	0.7
	Standard error . . .							
	2nd progeny	6	4226.5	175.2	24.1	6	357.3	11.8
	Average		406.0	12.0	1.6		51.0	2.1
	Standard error . . .							
			1.2	0.01	3.4**		1.2	2.9*
			Values of <i>t</i> for the comparison of the Bucks 152M and 633M					
			0.3		1.5			1.9

TABLE XII

Comparison between the performance of the third progeny and their dams

	Number of goats	Total milk yield oz.	Number of days in milk	Average milk yield per day oz.	Number of goats	Kidding interval	Average milk yield per day of kidding interval oz.
2nd progeny							
Average	4	4703.5	148.3	31.7	4	256.5	18.4
Standard error . . .		950.3	13.8	2.7		39.3	3.4
3rd progeny							
Average	8	5260.9	227.3	23.2	7	382.3	13.4
Standard error . . .		658.7	24.0	1.1		64.3	1.3
<i>t</i>		0.4	2.0	3.0*		1.4	1.5

TABLE XIII-A

Comparison of the performance of the foundation stock and the first progeny

	No. of goats	No. of lactations	Total milk yield oz.	Average milk yield per day of lacta- tion oz.	No. of goats	No. of lactations	Average milk yield per day of kidding interval oz.
Foundation stock	37	100	4679.8	26.4	27	69	16.2
First progeny	34	130	5832.5	33.1	30	110	20.9

TABLE XIII-B

Lactationwise comparison of the first, second and the third progenies

		No. of goats	Total milk yield oz.	Average milk yield per day of lacta- tion oz.	No. of goats	Average milk yield per day of kidding interval oz.
First Lactation	First progeny	32	5637.0	32.3	30	20.1
	Second progeny	28	4977.5	23.2	25	14.8
	Third progeny	10	4950.2	23.3	9	13.7
Second Lactation	First progeny	28	5797.5	35.4	23	21.0
	Second progeny	18	5395.4	27.2	16	16.4
	Third progeny	4	4858.0	25.0	4	14.7
Third Lactation	First progeny	19	7190.0	38.5	16	23.5
	Second progeny	10	5824.0	26.2	7	19.2
	Third progeny	4	2870.3	17.1	3	5.5

Having considered whether the sires were good transmitters or not, we shall now consider the question whether selection from amongst the dams would lead to improvement in the successive progenies. The consideration of this question has been made possible because no culling out of the female progeny was practised as a part of the policy of breeding followed on the farm. The whole of the progeny was kept and bred up. For this purpose,

the 19 mothers of the foundation stock were ranked in order of their performances in respect of the three characters over all lactations. The results of the comparison of the performances of the progeny of the first half compared with that of the rest are given in Table XIV. It will be seen that while the performance of the second group of 10 dams is significantly different from that of the first group of nine dams, their progenies do not

TABLE XIV

Effect of selection in the foundation stock

Character compared	Foundation stock dams				First progeny daughters			
	Number of goats	Number of lactations	Value oz.	Standard error oz.	Number of goats	Number of lactations	Value oz.	Standard error oz.
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Total milk yield oz.	First 9 Rest 10	35 33	5585.6 3807.6	215.3 265.1	15 19	68 62	5683.7 5995.7	286.5 310.3
<i>t</i>			5.1**				0.7	
Average milk yield per day of lactation oz.	First 9 Rest 10	38 30	30.3 22.8	2.3 2.0	16 18	63 67	32.8 33.5	1.9 2.1
<i>t</i>			2.5*				0.2	
Average milk yield per day of kidding interval oz.	First 8 Rest 8	25 23	22.1 12.2	0.7 0.9	14 16	56 54	21.5 20.4	1.3 1.0
<i>t</i>			7.9**				0.7	

show a significant difference in the performances. rately for bucks 48M and 49M has been shown in Table XIV-A and is seen to be similar. A similar

TABLE XIV-A

Effect on the progenies of bucks 48M and 49M of selection in the foundation stock

	Character compared (1)	Foundation stock dams			Standard error oz. (5)	First progeny daughters			Standard error oz. (9)
		Number of goats (2)	Number of lactations (3)	Value oz. (4)		Number of goats (6)	Number of lactations (7)	Value oz. (8)	
Buck 48M	Total milk yield oz.	First 5 Rest 6	17 21	5613.7 3595.1	391.1 352.6	7 7	33 22	6003.7 6493.3	456.7 579.6
	<i>t</i>			3.8				0.7	
	Average milk yield per day of lacta- tion oz.	First 5 Rest 6	21 17	29.9 21.2	3.3 2.2	8 6	39 16	35.1 33.9	2.4 4.1
	<i>t</i>			2.2				0.3	
	<i>t</i>			1.6				0.3	
Buck 49M	Total milk yield oz.	First 3 Rest 3	14 11	5281.6 3268.2	125.5 381.9	4 4	16 19	5586.0 4360.4	632.3 316.0
	<i>t</i>			5.5				1.8	
	Average milk yield per day of lacta- tion oz.	First 3 Rest 3	14 11	31.4 22.1	4.5 3.7	3 5	10 25	30.1 31.8	2.6 4.3
	<i>t</i>			1.6				0.3	
	<i>t</i>			1.6				0.3	

TABLE XV

Effect of selection in the first progeny

	Character compared (1)	1st progeny dams		Standard error oz. (4)	2nd progeny daughters		Standard error oz. (7)
		Number of goats (2)	Value oz. (3)		Number of goats (5)	Value oz. (6)	
First lacta- tion	Total milk yield oz.	First 9 Rest 9	6861.6 3931.1	380.9 257.6	17 10	4827.0 4927.2	444.0 636.8
	<i>t</i>		6.4**			0.1	
	Average milk yield per day of lactation oz.	First 9 Rest 9	35.7 28.5	1.2 0.9	13 14	27.3 27.7	1.6 1.0
	<i>t</i>		4.8**			0.2	
	Average milk yield per day of kidding interval oz.	First 9 Rest 9	24.6 16.9	1.2 0.5	12 13	14.7 15.0	2.0 1.3
	<i>t</i>		5.7**			0.1	
	<i>t</i>		4.8**			1.1	
Second lactation	Total milk yield oz.	First 7 Rest 8	6881.4 4398.8	521.3 180.1	7 8	5092.7 5325.6	411.1 514.1
	<i>t</i>		4.8**			1.1	
	Average milk yield per day of lactation oz.	First 7 Rest 8	40.7 32.3	1.7 0.1	7 8	24.2 29.8	1.6 1.6
	<i>t</i>		4.9**			2.5	
	Average milk yield per day of kidding interval oz.	First 5 Rest 6	26.7 20.8	0.9 1.6	6 6	16.5 15.1	2.4 1.9
	<i>t</i>		12.7*			0.4	
	<i>t</i>		12.7*			0.4	

comparison made with regard to the first and the second progeny, shown in Table XV, gives the same result. Both the tables show that selective breeding on the side of the dams is without effect and suggest that there is possibly no correlation between the performances of the dams and the daughters. This is actually found to be the case as will be seen from the values of the coefficient of correlation shown in Table XVI. Six of the nine values of the coefficient of correlation are negative and the remaining three are positive but all of them are small and non-significant indicating no real association between the performances of the dams and their daughters.

TABLE XVI
Coefficients of correlation between the performances of dams and daughters

	Total milk yield oz. (1)	Average milk yield per day of lactation oz. (2)	Average milk yield per day of kidding interval oz. (3)
Foundation stock and first progeny	+0.170	-0.270	-0.012
First progeny and second progeny, First lactation	+0.171	-0.005	+0.064
First progeny and second progeny, Second lactation	-0.102	-0.397	-0.206

The above analysis would indicate that the foundation stock had a low genetic variability. This suggestion is also supported by the fact that the values of the standard deviation of the three characters in successive progenies have not significantly changed. It is even possible to estimate approximately the amount of genetic variability present in the material by separating the total sum of squares into the differences between individuals, their lactations and the interaction, as shown in Table XVII. The extent to which the individuals have differential capacity to produce milk (when averaged over the three lactations) is

TABLE XVII
Analysis of variance of total milk yield of goats of the first progeny which have completed three lactations

Cause of variation	D. F.	Sums of squares	Variance
Between does . . .	16	135677631	8470852
Lactation order . . .	2	19106175	9553081
Residual . . .	32	141372459	4417889
Total . . .	50	296156266	

measured by the sum of squares 1, 35, 67, 76, 31 with 16 degrees of freedom. If there had been only random variation between individuals, this sum of squares would have been 4, 41, 78, 89 $\times 16 = 70, 68, 62, 24$. The difference between 1, 35, 67, 76, 31 and 70, 68, 62, 24 = 64, 99, 14, 07 is a measure of the variability between individuals excluding random variations and the ratio of this to the total sum of squares measures the extent to which the genetical factors affect the milk production. It can, therefore, be said that only about 22 per cent of the variance is ascribable to hereditary causes.

It is clear that the absence of improvement observed in the farm herd is mainly due to the unfortunate selection of both the purchased and the farm-bred bucks. If the bucks had been really good transmitters then even with low hereditary variability in the does, we should be justified in expecting improvement. But, as the results of progeny tests show, neither the original nor the farm-bred bucks appear to possess genes for distinctly higher level of milk production. There is a suggestion that in comparison with the worth of the other sires, sire No. 48M was probably a good transmitter; but the farm-bred bucks were definitely poor.

To conclude, the lack of distinct improvement is largely due to (i) the indifferent performance of the foundation bucks and (ii) the low genetic variability in the foundation dams. Periodical progeny tests would have revealed pre-potency of the different sires and suggested suitable changes in regard to their use. A study of the genetic variability in the successive generations would have revealed the potentiality for improvement of the material and also suggested suitable breeding plan. Another factor contributing to the lack of progress is the use of too few bucks to start with. In the absence of knowledge of the breeding worth of the bucks, the chance of locating superior individuals on conformation basis is necessarily small. Consequently it would have been desirable to start with a larger number in order to hold out a reasonable chance of securing a proved buck. The herd too was of small size while the mortality amongst the kids was high giving few corresponding lactations for conclusive comparisons. If culling had been practised, particularly on conformation basis and only partial records maintained, it would not have been possible to carry out the analysis on the generation basis which has been done here and thus assess the progress made during the last ten years.

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OBSERVATIONS ON THE TOXICITY OF RHODODENDRON ARBOREUM TO LIVESTOCK

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RHODODENDRON ARBOREUM grows abundantly in the Kumaun, Kashmir and Khasia hills between 4,000 and 11,000 ft. In January and February, when owing to frost and snow green herbage was scanty, a large number of animals, which had to subsist on hill-grazing, showed mild symptoms of intoxication and bulging eyes. Observations, extending over several days, indicated that these animals had to depend for nourishment to some extent on dry autumn-shed rhododendron

leaves. Believing the condition described above to be possibly due to the ingestion of these leaves, a systematic feeding experiment was conducted on two bulls, two sheep and two goats. These animals were of mature age and apparently in good health.

Leaves and flowers, including buds, were fed to the animals. The amounts given on different days are shown in Table I.

TABLE I

Dates	Species of animals	No. fed	Method of feeding	Material fed	Total quantity fed to animals (lb.)	Result	Remarks
17-11-38 to 18-11-38	Kumauni bulls	2	Voluntary feeding.	Leaf, flower and buds	About 14	All symptoms of poisoning	Sample 1
	Sheep	2	Do.	Do.	5	Do.	Do.
	Goats	2	Do.	Do.	3	Do.	Do.
19th to 21-11-38	Kumauni bulls	2	Do.	Do.	8	No symptom	Do.
25-11-38 to 30-11-38	Do.	..	Drenching suspension in water	Crushed leaf and flower in water	26	Do.	Do.
	Sheep	2	Do.	Do.	12 each	Do.	Do.
	Goats	2	Do.	Do.	..	Do.	Do.
27-11-38 to 30-11-38	Kumauni bulls fresh	2	Voluntary	Leaf, buds and flower	35	Do.	..
6-12-38 to 8-12-38	Do.	..	Do.	Do.	9 in two days	All symptoms of poisoning	Sample 1
	Old Kumauni bulls	2	Do.	Do.	7 in two days	Do.	Sample 1

SYMPTOMS

Bulls. After ingesting voluntarily about 14 lb. of the plant in two days, the following symptoms were observed:

Foamy salivation, vomiting, dullness, a staggering unsteady gait when forced to walk, crossing of the hind limbs, grinding of the teeth, bulging of the eye-balls and a disinclination for food. No febrile disturbance was noticed throughout the experiment. After two days of

abstinence, there was progressive abatement of the symptoms.

Sheep and goats. After consuming about 6 lb. of the plant, symptoms of frothy salivation, vomiting and grinding of teeth were observed.

TOXICITY AND ACTIVE PRINCIPLE

The tender leaves and the honey of rhododendron flowers are considered to be toxic [Kirtikar and Basu, 1933]. *R. californicum* and *R.*

maximum are reported to be fatally toxic in Oregon and the Alleghany mountains respectively [Lander, 1928]. The leaves contain a tanning resoluble by acids to yellowish-red rhodoxanthine. A reddish colour is obtained by treating the juice of the leaves with a mineral acid. The chemical nature of the toxic principle and its mode of operation are not yet understood.

A cow dead of rhododendron poisoning showed reddish tint in milk [Eve, 1907].

TREATMENT

Half the ailing animals were given an oleaginous purgative followed by gastric stimulants, such as nux vomica, gentian and ginger for three days. The treatment scarcely was necessary, as the untreated animals also recovered in much the same time, once further ingestion of the plant was stopped.

DISCUSSION OF RESULTS

As can be seen from the table, after two days' feeding all the animals evinced a disinclination for any more of the plant, though given nothing else to eat. Therefore for five days each animal was drenched with about 26 lb. of pulverized leaf and flower suspension in water. No symptoms of toxicity were manifested. Since it was possible that the bulls had developed a tolerance to this feed and also that there had been a change in the amount of the toxic principle in the five days' sample, the sample from the first-day locality was again utilized. After about 8 lb. of the plant were consumed by the same bulls in two days, all the symptoms of toxicity were freshly manifested. Two fresh bulls were fed over four days about 35 lb. of the second sample with no untoward symptoms, but, when

the first sample was fed to them as also to the bulls of the other group, and when only as little as 6-8 lb. were consumed in two days, all the symptoms appeared as markedly as before.

Thus we were led to conclude that it was not a question of tolerance but of the difference in composition of the two samples that had caused the failure to produce the symptoms of toxicity even when the animals were drenched. It is well known that climate, soil, season and the stage of growth affect the toxicity of a plant [Steyn, 1934]. In this case all things being almost equal, only the soil condition and the degree of transmission of toxicity to the progeny come into prominence, for the two localities were fairly distant. This finding seems to be in agreement with that of Steyn [1932] and Couch [1932].

SUMMARY AND CONCLUSIONS

Two samples of *rhododendron arboreum* from two different localities were used in feeding experiments on bulls, sheep and goats. Of these one sample was definitely toxic and the other was not. Soil condition is incriminated in the loss of toxicity of the second sample. The plant is not lethally toxic.

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MELANOMATA IN DOMESTICATED ANIMALS*

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(With Plates XIV and XV)

THE subject of melanomata has always been a vexed problem for the oncologist, the physiologist and the embryologist alike. Although a good deal of work has been done on the histogenesis of the specific tumour cell, the melanoblast and on the origin of the tumour itself, it has, if

anything helped to make the position more bewildering than before by introducing into the field several points on which considerable difference of opinion exists. Indeed, apart from those who believe in the existence of a specific neoplastic cell, there are those who claim that there is no such thing as a melanoblast, and that melanotic neoplasms are products of structural and functional changes in the ordinary cells of

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the body. In veterinary pathology the subject becomes more complicated on account of the unique predisposition of grey horses to melanomatosis—a point which needs elucidation in conformity with acceptable opinions on the subject.

In the collection of nearly 400 tumours at this Institute, only 13 (3.25 per cent) preserved specimens of melanomata are present: five from horses (4.5 per cent of neoplasms of horses), five from mules (14 per cent of neoplasms of mules) and three from bullocks (2 per cent of bovine neoplasms). Relevant information is wanting in connection with one specimen of each group.

Brief details of the source, location, naked eye and microscopical characters of these tumours are given in Table I. It will be realized that, with such limited material at one's disposal it is not possible even to attempt to solve any of the problems associated with the genesis of melanomata. In this article it is, therefore, proposed only to record the incidence of the tumour in this country*, and to give such clinical and microscopical observations as seem to be of interest. A general review of available literature on the subject is also included.

*The incidence has been calculated from the records of this Institute for the past sixteen years.

TABLE I

A summary of the study of melanomata of animals in the collection of the Institute

Path. specimen number	Animal	Colour	Age	Location of tumours etc.	Microscopical examination	Microscopical diagnosis (benign or malignant)	Remarks
1010/1926	Horse	Grey	Aged	Skin of tail and anal region.	Small and large, deeply-pigmented	Benign	
1070/1930	Horse	Grey	16 years	Lower side of tongue	Deeply-pigmented	Benign	The only tumour detected and removed. Normal recovery.
79/1934	Horse	?	?	Ulcerating skin of knee	Do.	Benign (Plate XV, Fig. 1)	
239/1931	Horse	Grey	Aged	Perineal region, head, neck, sheath (Plate XIV) and abdominal cavity, peritoneum and tail	Do.	Malignant	
266/1937	Horse	Grey	Aged	Prepuce, liver, lung, ? spleen	Small and large, deeply-pigmented.	Benign	
1116/1927	Mule	Grey	8 years		Small and large, superficial, deep brown to black tumours in liver	Malignant	Some galls of blood-stained fluid in abdominal cavity
1121/1927	Mule	Grey	15 years	Withers, thigh, pleura, heart, lung, liver, peritoneum	Lightly-pigmented, grape-like, pleural nodules	Malignant	Progressive debility. Slaughtered
46/1934	Mule	Grey	18 years	Thoracic tumour, affecting lung and pericardium	Deeply-pigmented	Malignant	
81/1935	Mule	Brown	10 years	Between thighs, with extension to udder	On udder, scirrhous tissue with lightly-pigmented rounded lesions	Malignant (Plate XV, Figs. 2, 3).	Loss of condition. Constant blackish discharge from teats
232/1935	Mule	?	?	Thigh	Deeply-pigmented	Malignant.	
958/1926	Bullock	Black	7 years	Skin of chest	Appearance of "Calcutta Sore". Underlying tumour deep-brown.	Benign. Associated with filariasis (Plate XV, fig. 3).	
153/1935	Bullock	?	8 years	Scrotum	Deeply-pigmented	Benign	
184/1937	Bullock	Black and white	5 years	Pastern. tumour	Podomelanotic	Benign	Surgically removed with success

The five cases in horses are from aged grey animals mostly over 16 years of age. As is well known, a grey horse with advancing age is very likely to develop melanomata. In fact, it is claimed by many that no grey horse, if allowed to live long enough, will escape melanomatosis. This high incidence is explained by some as being due to a gradual loss of pigment (melanin), as the

animal turns from grey to white with advancing age. Normally, in man and animals, there is a steady loss of pigment from the skin during the process of continuous desquamation. In animals, the casting of hairs is an additional factor, involving loss of melanin. In coloured animals, the demand for the pigment is kept up throughout life. In the grey horse, however, this demand goes on

decreasing, particularly after middle age, so that much melanin remains unutilized. Under the circumstances, according to van Dorssen [1930], the cells containing and manufacturing melanin undergo hypertrophy and hyperplasia, the process ending up as an uncontrolled multiplication of cells as in neoplasms. The frequency with which primary melanomata arise near the root of the tail is explained by M'Fadyean [1933] as possibly due to decline in demand for melanin in the long hairs of the tail. Whatever the cause may be, the fact remains that the grey horse is singularly predisposed to melanomatosis. There is no such parallel in tumours of man or of animals, even grey, except apparently in the case of the grey mule and the white goat [Jackson, 1936]* This predisposition must be inherited, as certain records suggest (Virchow), [cited by Ewing 1934, and Feldman 1932] and according to M'Fadyean may have originated as a mutation.

The most common sites for primary melanomata in the horse are the preincum and the ventral surface of the tail (Plate XIV). As a rule, the tumours are, in these situations, not single but multiple. Less commonly, melanomata, apparently primary, may occur elsewhere on the skin, e.g. on the head, neck, scrotum, udder, sheath and limbs, and perhaps in some cases in the deeper tissues and internal organs. One of the specimens in our collection was recorded from beneath the tongue.

The tumours are not always malignant; however, in some equine cases, under the influence of some unknown factor or factors, a benign growth may suddenly assume malignant characters and metastasize widely and rapidly, via the lymphatics, and later on, perhaps also via the blood, infiltrating the regional lymph glands, serous membranes and internal organs, even including the brain, nerves, blood vessels, bones and heart; somewhat rapid cachexia follows, causing death or rendering destruction more economical. Several workers consider every melanoma as a potentially malignant tumour and, in medical pathology, every pigmented mole, naevus or wart as a potential source of melanoma.

The tumours are more or less pigmented. The pigment may vary in different tumours, or in different parts of the same tumour, or within a group of metastatic growths.

The cells of deeply pigmented tumours are usually large in size and may occasionally assume, by fusion or independent nucleus division, the appearance of giant-cells. The nucleus is small,

pale, more or less oval and usually excentrically situated; altogether, the picture suggests that the nucleus has undergone hypochromatosis and has been displaced by an excessive accumulation of melanin. The aggregation of pigment must obviously be responsible for the large size of the cell. In extremely pigmented tumours, the cells, in depigmented sections (Plate XV, fig. 1), resemble some what the cells of sebaceous glands and appear to have lost their staining affinities and perhaps their normal function. These points are brought home at once if one compares the picture presented by melanoblasts of a malignant growth (*vide infra*). Excess of pigment must naturally escape outside the tumour cells to be taken up by phagocytes, or deposited in the surrounding connective tissue.

Different from the above picture is that of a malignant melanoma. The cells are smaller, often not so deeply pigmented, or at places even unpigmented. The nucleus and cytoplasm retain their normal staining properties. The difference between these cells and those described above appears essentially to be one of physiological differentiation, the cells of the malignant melanoma exhibiting the tendency towards multiplication rather than towards pigment-formation. Mitotic figures are, therefore, easily seen (Plate XV, fig. 2). The cells tend to be rounded, polyhedral or spindle-shaped and may or may not show an alveolar arrangement.

It should be borne in mind, however, that it is difficult to assess the degree of pigmentation either by the naked eye or microscopically and that tumours described as deeply pigmented may possess malignant propensities. Thus, malignancy depends not on the amount of pigment but on the presence of a fair proportion of proliferative rather than melanin-producing melanoblasts. It is reasonable to suppose that this proportion bears a direct relationship to the degree of malignancy.

Local connective tissue response appears to be relatively delayed in melanomata and a true stroma may be absent, at least in the early stage of the growth. Deeply pigmented, clinically benign tumours of the skin contain at first much of the local connective tissue. Histologically, such tumours only show accumulations of melanoblasts lying in the meshes of a connective tissue network. The pigmented areas, having at first a diffuse border, gradually become well separated from the surrounding tissue as a rounded focus, the adjacent tissue more or less stimulating a capsule. In the case of malignant tumours, signs of invasion of the surrounding tissue (Plate XV, fig. 3) and of metastasis can be observed without much difficulty also; in such tumours the connective

*One may, however, note that MacCallum [1932] discovered ten melanomata, all in white men, in 12,000 autopsies wherein the proportion of Negroes was high. Ewing [1934] feels that the rare incidence of the tumour in Negroes may be due to difficulty in diagnosis.



FIG. 1. Pony 366 from which Spec. 239/1934 was removed. Note tumours on tail, sheath and perineum.

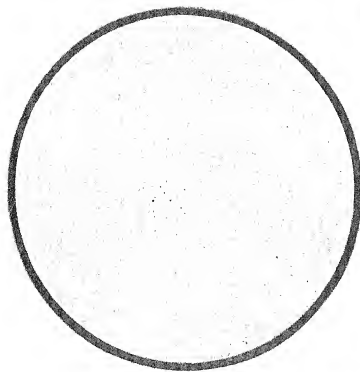


FIG. 2. (Spec. 79/1934). $\times 138$ Depigmented cells of a benign tumour. Note large bloated cells with small pale nuclei.

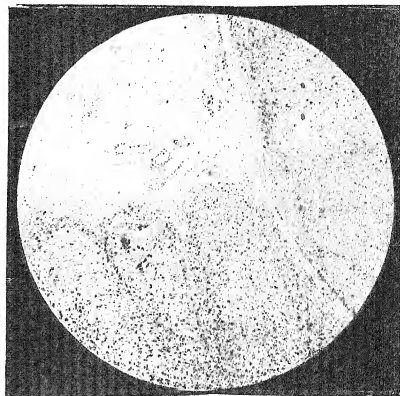


FIG. 4. (Spec. 81/1935). $\times 30$ Periphery of tumour. Note growth by invasion into surrounding tissue.



FIG. 5. (Spec. 958/1226). $\times 24$ Partly depigmented micro-section. Note 3 worms in section in deeper epidermal layers, and tissue reaction and melanoma-formation in the cutis.

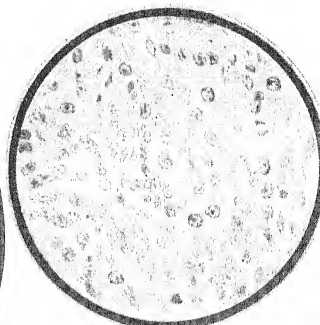


FIG. 3. (Spec. 81/1935) $\times 131$ Malignant tumour with normally staining cells containing only slight pigment. Numerous mitotic figures.

tissue of the affected site reveals an early reaction sufficient to be recognized as a true stroma.

Microscopically, the stages between the benign and malignant melanomata can often be seen. Suitable material will also illustrate the possibility that under unknown circumstances a benign-looking neoplasm may assume malignant characters. Both types of melanoblast may co-exist in the same microscopic field or in different parts of the same section, suggesting a proliferative tendency supervening on the essentially primary function of pigment-production.

The tumours may attain large dimensions and vary in consistency from fluid or pulpy to hard; accordingly, on incision, the amount and nature of the escaping pigment varies. Portions of melanotic tumours kept in commonly used fixatives often impart the melanin tinge to the preservative. Superficial tumours are likely to ulcerate and discharge a dark-pigmented, foul-smelling fluid (79/1934). Rarely, the formation of the pigment may be so excessive that it may appear in the blood stream (melanaemia) in the urine (melanuria) or in various other situations (melanosis). The primary or metastatic tumours in the serous cavities may be responsible for the presence in these cavities of pigmented fluid, sometimes amounting to several gallons.

In the horse, the cutaneous tumours are nodular, plain or elevated, occasionally pedunculated growths covered with skin. The tumours in the internal organs are generally looked upon as metastases of a known or undetected primary skin-tumour. According to some [Ewing, 1934], primary melanomata, especially in man, may grow in these situations, owing to the misplacement of melanoblasts during development.

It should not be understood that horses other than grey are immune from melanomatosis, for veterinarians do occasionally encounter such cases.

Few cases of melanomata in mules have been recorded. In the collection at this Institute, there is material from five cases, but generalization is not possible on the strength of such meagre data. Of the five, four were accompanied by details regarding colour and age; three were from grey aged 8, 15 and 18 years, the fourth being from a 10 year-old brown animal. All four specimens were malignant tumours, which fact is in conformity with the observations of other workers [Anbry, cited by Feldman, 1932; Mathews, 1929; Frank and Morrill, 1937].

Unlike what occurs in the horse, the perineum and tail in mules do not seem to be the common sites of primary melanomata. The tumours in our collection affected the deeper tissues of the shoulder and thigh region, the organs and wall

of the thorax and the abdomen, and in one case the mammary gland. It would appear that, whereas in the horse the apparently benign and superficial tumours are the first to attract attention, in mules early malignancy with the involvement of the internal organs is frequent. Indeed, in these animals, numerous metastatic growth may be discovered in the internal organs at autopsy, and the primary growth may have escaped detection during life. In one of the cases in our collection (1116/1927), it was suspected that a kick had been responsible for provoking a primary tumour in the sheath, discovered and interpreted as such on autopsy, to malignancy and internal metastasis, causing death within three months.

The three specimens of bovine melanomata in our collection came from the skin of the chest, scrotum and pastern and were clinically benign and operable. In one of them (958/1926), the skin showed the evidence of filariasis (Plate XV, fig. 4), but what relation, if any, this had with the presence of the melanoma is open to conjecture. The tumours were nodular, hard and more or less deeply pigmented masses. One of them was pedunculated. There seems to be no age or colour predisposition to melanomatosis in bovines, although Funniger [cit. Feldman, 1932] has observed the occurrence of melanotic tumours more frequently in albino than in dark cattle. Malignant melanomata are rarely found in bovines, (vide specimen No. 115/1938, Addendum). Unlike melanomata, melanosis, i.e. mere dark brown or black pigmentation of the tissues or organs without any material dysfunction of the parts or of the organ, is relatively more common in cattle. In calves, melanosis of the cutis (*melanosis ncutiosa*) is seen; this, unlike the congenital uaei of man, tends to disappear with age (Feldman, 1932).

Although not represented in our collection, melanomata also occur in animals other than equines and bovines. In swine, apart from patchy benign melanosis of the skin, the occurrence of melanomata, often malignant with widespread metastases, has been noted, the primary lesion appearing usually in or under the skin, not infrequently when the animal is only a few weeks old. It is suspected that in certain breeds heredity plays a part [Caylor and Schlotthauer, 1926]. The tumours in adult swine are usually benign.

The dog, like the pig, is also subject to melanomatosis as well as simple melanosis. According to Gray [1900] certain breeds of dog, particularly the Yorkshire terrier, are predisposed to the former, the tumours being of the nature of cutaneous elevations, or of single, stalked or sessile smooth

warts growing usually on the sides of the trunk or on the feet or prepuce. Recorded cases show that severe generalized melanomatosis occasionally occurs in the dog, involving the skin and internal organs, but at times excluding the liver. Rarely, the central nervous system may also be involved [Feldman, 1932]. Melanosis is seen in the subcutis, lungs, liver, kidneys, etc., the pigment being present in the tissue spaces or in phagocytes.

Whether melanosis in the pig and dog is capable of stimulating the growth of melanomata is not clear, but such a possibility would appear to exist. Jackson's cases [1936]—five canine and two porcine—"had much the same morphology as the human melanotic skin tumours ranging from 'naevi' to definite anaplastic neoplasms". It was not possible, however, to classify them as melanocarcinoma or melanosarcoma. Jackson's porcine cases were distinctly different from epitheliomatoid melanomata of Duroc Jersey pigs, encountered by Caylor and Schlotthauer [1926].

Melanomata have been described in white goats in South Africa and are known to affect mainly, but not exclusively, the Angora goats. Their incidence may be gauged by the fact that they form approximately 40 per cent of all caprine tumours at Onderstepoort [Jackson, 1936]. While regarding the caprine material as 'an unrivalled wealth of specimens of the study of what are usually regarded as typical melanotic epitheliomas', Jackson refrains from definitely attributing an epiblastic origin to them. Thomas [1929] has classified these tumours as basal-cell melanotic epitheliomata. In the list of melanomata at Onderstepoort are also included one acanthoma of the horse and a basal-cell epithelioma of the cat.

Melanomata have also been described from the rabbit [Brown and Pearce, 1926], cat [Mulvey, 1906], and chicken [Goldberg, 1919; McGowan, 1928]. McGowan's cases, in the fowl, included one affecting the ovary, which, unlike the rest (mesoblastic) of his specimens, was considered by him to be of epithelial origin.

Histogenesis. It is generally agreed that melanomata result from the unchecked proliferation of a specific pigment-producing cell, the melanoblast, which is responsible under normal conditions for supplying pigment for physiological purposes. The neoplastic (malignant) melanoblast is capable of metastasizing in the same way as cells of other malignant tumours.

The pigment, melanin, is not derived from the blood but is a special metabolite of the cell and is contained in or outside the nucleus. It is probably formed by the action of an oxidase contained in the melanoblast upon a melanogenic protein

substance resembling, or related to, tyrosin. Bloch's 'dopa' reaction involving the use of an extract of broad bean containing dioxypheylanin is well known for demonstrating the ferment. The substance is related to tyrosin, tryptophane and adrenalin, and is similarly oxidized to a dark brown pigment resembling melanin. The reaction is given by certain cells of the epidermis, blood and bonemarrow and by the cells of non-pigmented melanomata. According to MacCallum [1932], the ferment is not demonstrable in the cells of the cutis, some of which (melanophores) may contain melanin, not elaborated by them, but merely derived from the epidermal melanoblasts. This, by the way, indicates the passage of melanin from the epidermis to the dermis.

The origin of melamins by the action, as suggested by the 'dopa' phenomenon, of an oxidase upon an oxidizable chromogen, perhaps allied to tyrosin, is helpful in elucidating the bronzing of the skin in Addison's disease wherein the precursor (tyrosin) of adrenalin is said to be oxidized in the skin to form a brown pigment [MacCallum, 1932]. Gessard [1902] detected the presence of tyrosin and tyrosinase in equine melanoma. Even so, the usual presence of sulphur in natural melanin remains unexplained.

Oxidase is entirely absent from the skin and hair of albinos or in leucodermic patches or white hair; but when present in less amount may be increased by the use of actinic light ('tanning').

The terms 'melanocarcinoma' and 'melanosarcoma' are often used to indicate the histological resemblance of the tumour to epithelioma and sarcoma, respectively. The terms should, however, be discontinued as they are suggestive of the histogenesis of the neoplasm, which is anything but settled even today. Further even, from the point of view of structure, the existence of demonstrable intermediate stages convinces one at once that the expressions are artificial. Some melanomata of the human skin and nervous system may resemble peritheliomata in their microscopical structure. Feldman [1932] prefers to classify melanomata simply as benign and malignant.

The origin of the melanoblast is undetermined, and a large number of hypotheses has been put forward by various workers. The observations made have been mainly based on factors such as the development of colour function in the animal kingdom, the structure of the naevus, the nature, morphology and situation of pigmented cells and their association with blood vessels and nerves. It may be said at once that, unless the melanoblast has a multiple origin and perhaps an inconstant morphology, the diverse conclusions

arrived at by different authors would be difficult to reconcile.

From the standpoint of histology, melanoblast are suggested to be 'highly differentiated connective tissue cells which arise early in the ontogenic development from mesenchymal cells' [Maximow and Bloom, 1935]. Two types of melanoblast are recognized in the skin, distinct from the dermal melanophores which are situated in the superficial layers of the dermis, in large numbers at places (e.g. around the anus), and which contain coarse uneven pigment-granules not formed by them, but perhaps received from the epidermis [Post, 1894; Ewing, 1934]. Of the true melanoblasts, elaborating and containing small and uniform pigment granules and giving a positive 'dopa' reaction, one set consists of branched cells—epidermal melanoblasts*—situated just under the basal epidermal cells between which their out-growths project, while the second set consists of dermal melanoblasts and is known to occur in the skin of apes and in the scaral region of the newborn Mongol ('Mongolian Spots', 'Blue Naevi'). The melanin in the basal layer of the epidermis would appear to be derived from epidermal melanoblasts, for the former do not give a positive 'dopa' reaction. Yet, a large number of workers on the subject holds that they are pigment-producing cells and hence true melanoblasts. Such an opinion was held as early as 1894 by Post and subsequently by a long line of observers including Cowdry [1932, 1934]. The conciliatory suggestion that the epidermal 'dopa'-positive melanoblasts are derivatives of the basal cells seems unlikely in view of Bloch's observations on foetal epidermis which indicate that the former are migrated mesoblastic chromatophores and that they give rise to the tactile cells of Merkel-Ranvier's corpuscles and probably constitute the melanin-producing component of pigmented naevi which, according to some authors, develop from the end-organs of the sensory nerves of the skin (corpuscles of Meissner and of Merkel-Ranvier).

Pigmented cells ['Melanophores' Maximow and Bloom, 1935; pigment-producing true melanoblasts, Ewing, 1934; and Arey, 1932] are also present in the choroid of the eye—one of the sites of melanomata in man—and in certain positions in association with the nervous system. According to Ewing [1934], 'the mesoblastic origin of the choroidal chromatophores must be conceded', and Arey [1932] is of opinion that these cells are 'true melanoblasts and can revive this potentiality

when stimulated pathologically'. Recorded cases in human beings, however, tend to indicate that melanomata of the eye may arise not only from the choroidal elements but also from the pigmented retinal epithelium [Ewing, 1934].

Ewing [1934] declares that melanomata are derived 'from a specific mesoblastic cell, the chromatophore, probably also from tactile cells lying in the epidermis and probably also from nerve cells in the derma' and thus summarizes the position, obviously uncertain, regarding the histogenesis of melanomata. In determining the origin of the melanoblast various workers have concentrated upon the congenital naevus a potential fore-runner of melanoma in man. This has, naturally, not been possible in animals wherein naevi are not so common and their behaviour as melanoma-producers is not so well understood. Jaeger [1909] in 63 autopsies on grey horses failed to discover pigmented naevi of the skin.

The histology of the naevus often suggests an epidermal rather than a mesodermal derivation. Unna [1893] suggested, on strong evidence, the epidermal origin of naevus cells by metaplasia of groups of epidermal cells a view which has attracted several followers. Among them, Dawson [1925] holds that the basal epidermal cells are true melanoblasts which may migrate into the dermis to form naevus cells, since bridging strands of cells between the naevus cells and the epidermis have been repeatedly demonstrated. That ectodermal cells are capable of producing melanin is further supported by the pigmented cells of the retina and by the occurrence of undoubted melanotic epitheliomata in man [melanotic whitlow, Hutchinson 1886] and animals [McFadyean, 1890; McGowan, 1928; Jackson, 1936]. It must be admitted, however, that such tumours are rare. While Post [1894] claims pigment formation in mammals as the almost exclusive function of epithelial cells, and while as histological studies of naevi and early melanomata point towards the conclusion that 'epidermal pigment arises in the epithelial cells' [Ewing 1934], there are others [Ehrmann, 1885; Achy, 1885] who consider that epithelial tissue can form no pigment but may acquire it from wandering pigmented connective tissue cells.

Others [Pini, 1902; Rieke, 1903; Jaeger, 1909] look upon naevus cells as modified fibroblasts, i.e., derived from mesodermal elements.

Pigment formation, at least in the lower mammals, is recognized by many to be essentially a function of specific mesoblastic cells, and it seems certain that both in man and animals most, though not all, melanomata are of mesodermal origin. McFadyean [1933] interprets the characteristic

*These are often referred to as Langerhans' stellate or dendritic cells. Maximow and Bloom [1935] apply the term to altogether different structures in the epidermis and doubt whether these are cells at all.

tolerance of the tissues towards the melanin-forming cells' of melanoma as a point perhaps in favour of a mesoblastic origin of the tumour. At the same time it appears certain that in dealing with melanomata in man, the elaboration of pigment by epiblastic elements must also be considered [Ewing, 1934].

It is suggested [Ewing, 1934; Maximow and Bloom, 1935] that the pigment-forming cell arises very early in the formation of the mesenchyme and exhibits specific properties, some of which resemble those of epithelium, and that this would explain the tendency of some melanomata, the so-called melanocarcinomata, to manifest an epitheliomatous histology. Ribbert [1897] and others are inclined to attribute all naevi and melanomata to aberrations of the mesoblastic melanoblast. According to this view, the pigment in the epidermis is derived from the dermal melanoblast (mesodermal chromatophore) and the formation of naevi and cutaneous moles would follow excessive deposition of the pigment in the skin, leading to proliferation of epidermal cells with pigment formation [Ewing, 1934]. Summing up the contention between the epi and mesodermal origin of the chromatophore, Ewing says 'the histological data appear to me strongly, but not decisively, in favour of the epithelial origin, while the theoretical considerations are all against the epithelial theory'.

The suggestions of some earlier authors [Denierville, 1830; Ziegler, 1908; Jaeger, 1911] regarding the endothelial and perithelial origin of naevus cells has found little support.

Recklinghausen [1882] and subsequently others, have produced strong evidence to support the contention that pigmented naevi and neurofibromatosis are related conditions. Nerve fibrils have been detected in groups of naevus cells by Söldan [1899], and by Masson [1926] who has shown that naevi and melanomata arise from cells of the end-apparatus of cutaneous sensory nerves (Meissner's and Merkel's corpuscles) and that the two should be considered as disorders of the nervous system. The cells actually proliferate in the neoplastic process, but their origin is not definitely known. Masson's findings are expressed in the following quotation from Ewing [1934]. 'In the derma, the naevus cells come from the cells of Meissner's corpuscles, in the epidermis, they come from the cells of Merkel-Ranvier, and from the chromatophores, in both situations. Masson does not attempt to decide whether the cells involved are mesodermal, glial, or epidermal'.

Whereas in the opinion of certain authors, the evidence favours the epithelial origin of the melanoblast [MacCallum, 1932], others, not with-

out reservation, consider this cell to be of mesoblastic origin [Beattie and Dickson, 1926]. But the view, in keeping with our present knowledge, should be the one expressed by Ewing [1934] Pigmented cells from melanotic sarcoma have been shown to reveal in tissue cultures [Grand, Chambers and Cameron, 1935] characters consistent with a mesenchymal origin. But this by no means precludes the possibility, not yet exhaustively investigated, that cells taken from melanotic carcinoma will reveal epithelial characters in tissue cultures. It may be stated here that Caylor and Schlotthauer [1926] were unsuccessful in their attempt to transplant porcine melanotic epitheliomata.

The position with regard to equine melanomatosis is far from satisfactory. The work of van Dorsen [1903] on the histology of equine skin shows that, in coloured horses of all ages, the epidermis is pigmented and the cutis devoid of pigment-cells, but that in aged grey horses, the opposite is the case. Unfortunately, information on the situation of pigment in the skin of grey foals is not available for comparison. Van Dorsen suggested that the pigment is conveyed to the epidermis by dermal (i.e. mesoblastic) melanoblasts and that in aged grey horses the demand for pigment is so much diminished that it accumulates in the cutis and, following the upset in pigment-metabolism, the growth of melanomata results (as mentioned earlier). On the contrary Schwalbe [1893] found nothing to indicate the transport of pigment from cutis to epidermis. Virchow views the frequency of the tumour in grey horses as an evidence of congenital dyscrasia, although why this should be so particularly in the grey horse is not explained.

Whatever the origin of the melanoblast, the question still remains. What is it that transforms it into a neoplastic cell? The history of continuation in one of our equine melanomata (1116/1927) and the discovery of filariasis in a bovine tumour (958/1926) may, however, have interest in view of the statement of Kettle [1925] that in three of his cases in man a history of previous injury by a rusty nail was associated with the development of melanoma on the foot, in the absence of a pre-existing mole. Yet, one finds it difficult to explain the notorious susceptibility of aged grey horses to melanomatosis on the theory of chronic 'local' irritation, of toxæmia* or of microparasitic invasion, for these factors must operate equally in

*Since writing, a paper has appeared by Hadwen [1937] wherein he mentions that he has succeeded in producing growth of black hair around needle-punctures in rabbits inoculated with diptheria toxin. He suggests that injuries such as saddle-galls and insect-bites may induce a de or hyper-pigmentation of the skin.

horses other than grey. Apart from possible 'racial' causes, hinted at by Virchow as 'congenital dyscrasia' and by M'Fadyean [1933] as 'predisposition by mutation', there exists another suggestion: Post [1894] incriminated injurious substances produced locally by melanotic cells for provoking a neoplastic response on the part of these cells. Eppinger [1910] regards 'the excess of pigment and its products, especially indol and skatol, as the cause of overgrowth of the cells'.

SUMMARY

1. An account is given of specimens of melanomata of domesticated animals accumulated in this Institute. Their general histological structure is indicated and, where available, the clinical records and autopsy findings have been added.

2. No attempt is made to elucidate the problem of histogenesis of these tumours but only a general review of the available literature is given.

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Addendum

Since writing, a specimen of bovine malignant melanoma was added to our collection (Spec. 115/1938). The material was from a cow said to have died of generalized multiple melanomatosis. The history of the animal was that it had developed, about a year before death, a 'tumour-like ulcer' on the hind quarter of the udder, which healed up in a few days. Six months later, the cow calved and after two months the lesion re-occurred, rapidly becoming large and producing febrile symptoms with progressive emaciation and dyspnoea. The cow was operated on for 'abscess'. On opening the lesion, a yellowish fluid escaped and a soft tumour with very deep extensions was removed. The patient died the next day and revealed at autopsy multiple black nodules in all internal organs, especially in the lungs, liver and kidneys. The colour and age of the cow could not be ascertained.

SELECTED ARTICLES

BOVINE TUBERCULOSIS IN THE TROPICS WITH SPECIAL REFERENCE TO UGANDA, PART II*

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EXPERIMENTAL TUBERCULOSIS IN ZEBU CATTLE

In part I† of this paper attention has been drawn to the very low incidence of tuberculosis

in Zebu cattle in India and Tropical Africa. This remarkable paucity of tuberculosis does not, however, apply to all cattle in warm climates, for in Uganda there is the Ankole breed of cattle in which tuberculosis has proved to be extremely widespread.

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† *Journal of Comparative Pathology and Therapeutics* Vol. 52, Part 4, pp. 322-335, December, 1939.

The environmental conditions appertaining to cattle husbandry in the tropics as compared with colder climates has on occasions been brought forward to explain these circumstances [Hornby, 1934], but, as pointed out earlier, this cannot be accepted as the true explanation. The tubercle bacillus as found in Zebu cattle has been said to be of lower virulence than normal [Sheather, 1921], but when the literature is discussed it will be seen that there is little evidence to support this contention, whilst in Uganda numerous bovine strains have been isolated from infected cattle and all have proved to be of standard virulence.

Lastly the question of race resistance has been investigated and it appears it is here where the true explanation lies.

Experimental evidence points to the fact that Zebu cattle possess a considerably greater resistance to tubercular infection than those of European breeds in so far as standard European strains of the tubercle bacillus are concerned.

LITERATURE

Liston and Soparkar [1917], referred to in part I of this paper, concluded as the result of their experiment that the comparative infrequency of the disease among cattle in India is due to natural resistance rather than to any method of housing or keeping cattle in India as compared with England. They also point out that whereas a dose of 50 mg. of a culture of tubercle bacillus when injected into English calves invariably causes severe generalized tuberculosis, this does not apply to Indian calves, where there appears to be a much greater individual variation in resistance.

Sheather [1921] carried out inoculation experiments on buffalo and cow calves, using a local strain of tubercle bacilli isolated from a tuberculous bovine. Five buffalo calves received 10 mg. of culture subcutaneously and five received 50 mg. Twelve cow calves were similarly inoculated, half receiving 10 mg. of culture and half 50 mg. None of these animals died of the inoculation and they were killed off between 152 and 159 days later.

All animals put on weight and the post-mortem examinations were classified as follows:

(a) Fourteen animals

Slight tuberculosis.

A lesion at the site of inoculation.

Lesions in the thoracic glands and possibly abdominal glands.

A few lesions in the lungs or spleen.

(b) Five animals

Extensive but not severe.

As above, but with the addition of tuberculous pleurisy or peritonitis or both.

(c) Three animals

Generalized but not progressive. Cases in which there was evidence of invasion of the organs by way of the blood streams with the production of numerous lesions in the lungs.

The results are compared with those of the Royal Commission on Tuberculosis (2nd interim Report, Part II Appendix, Vol. I, p. 34), which showed that a subcutaneous dose of 50 mg. of culture almost invariably produced fatal generalized tuberculosis within seven weeks and in the case of a 10 mg. dose 60 per cent died within eight weeks.

The author concludes from his experiments both on rabbits and on cattle that the virulence of the tubercle bacillus isolated from Indian cattle is lower than that isolated from cattle in Europe. He states:

'Glen Liston's experiments indicated that Indian cattle are less susceptible to infection with the tubercle bacillus than English animals when tested with a virus of European origin.'

'The experiments here recorded indicate that this is not the sole or possibly even the most important factor in determining the comparatively frequent occurrence of tuberculosis in Indian cattle. They appear to indicate beyond all possibility of doubt that the strains of tubercle bacilli affecting cattle in India possess a distinctly lower degree of virulence than tubercle bacilli isolated from cattle in Europe.

'A point which would appear to support the view that the lower virulence of the organism is the more important factor is that in practically every instance the natural lesions which have come under observation have been restricted to a few glands and have been to a very large extent calcified.'

Soparkar [1926] carried out a large-scale experiment to test the susceptibility of various Indian breeds of cattle to tuberculosis. A type strain obtained from the National collection was used in a dose of 50 mg. inoculated subcutaneously. One hundred and forty-one calves were used in the experiment, and the results showed that whilst a considerable number (48) proved very resistant and remained well and lived beyond 90 days, the susceptibility varied considerably. Ninety-three died of acute generalised tuberculosis in periods varying from 17 to 101 days. Apparently out of these 93, only 10 showed the very severe type seen in English calves.

The author summarizes the position in the following paragraph:

'While the experiments of the Royal Commission on Tuberculosis show that a dose of

50 mg. of culture of bovine origin given subcutaneously is almost invariably sufficient to overcome the resistance of English (Jersey) cattle, and, with rare exceptions, produces generalized tuberculosis of a severe type which proves rapidly fatal, the results of the present experiments would indicate that a certain proportion of Indian calves equally small in size remain well after similar infection, and, when autopsied, show slight or minimal lesions, indicating that they are possessed of very high powers of resistance to tuberculosis as compared to English calves.

Soparkar [1927], in a paper dealing with the virulence of tubercle bacilli isolated from cattle in India, reviews Sheather's [1921] results and quite rightly draws attention to the fact that Sheather used cultures which were six months old, which of course detracts considerably from the significance of his results.

Soparkar carried out inoculation experiments on rabbits and calves, using both European and Indian strains of tubercle bacilli. Of the European strains actively growing young cultures were used, also cultures which were six months old. Of the Indian strains only young cultures were used.

It is difficult to draw definite conclusions from the results, but it is quite evident that the European strains six months old were not by any means as virulent as the young cultures, either in rabbits or calves. It is unfortunate that six months old Indian strains were not used in order to make a direct comparison with Sheather's work.

Two series of calves were inoculated, one with 21-day-old European cultures (Table V), and the other with Indian cultures of the same age (Table VII).

On examination of Table V, *i.e.* the inoculations with European cultures, 13 of the 24 calves died in an average period of 40 days whilst Table VII, *i.e.*, the inoculations with Indian strains, shows that 15 calves died out of 19, but the average period of survival was 71 days.

It is difficult to say on these results whether one strain is more or less virulent than the other. However, a number of calves inoculated with the Indian strains did, in fact, die of acute generalized tuberculosis in the same way as those inoculated with a known virulent strain of European origin. This is important and indicates that the Indian strain was not of markedly low virulence, and that individual differences existed in the subjects inoculated.

It will be recalled that none of Sheather's [1921] calves died, and when killed they only showed local lesions. All the Indian strains

proved highly virulent for rabbits in the same manner as the type European strain.

Soparkar's findings are as follows: "The rarity of gross tuberculous lesions hitherto observed among indigenous cattle and the usual tendency of the lesions to remain localized in affected animals, cannot therefore be ascribed to any noteworthy difference in the virulence of the infecting organisms."

It must be remembered that the indigenous cattle of India are Zebu in type.

Experimental

The object of these experiments was to ascertain how Zebu calves reacted to a test dose of virulent bovine tubercle organisms with a view to discovering whether any resistance to tuberculosis did in fact exist in this breed of cattle. The opportunity was also taken to test calves of the Ankole breed for comparative purposes.

Technique. The dose of organisms was fixed at 50 mg. (Royal Commission on Tuberculosis) and was given subcutaneously. All calves were previously tested with tuberculin by the double intradermal test and only non-reactors used in the experiment. The strains used were B.V. 28 and B.V. 34, kindly supplied by Dr. A. S. Griffith of Cambridge as being typical dysgonic virulent bovine cultures of recent isolation. The strains were highly virulent for rabbits in doses of 0.001 mg. intravenously and 10 mg. subcutaneously.

The cultures were grown on inspissated ox serum and only young, vigorous cultures, not more than 10 days old were used. As controls to the actual injections of the calves, rabbits and/or goats were inoculated with appropriate doses of the same cultural growths.

It was intended to kill off all surviving inoculated calves after a period of 90 days and this was done in a number of cases, but later the survival time was allowed to extend, so as to ascertain in what manner the tissues of a resistant calf would react to a tubercle infection, and perhaps enable the degree of resistance to be more accurately assessed.

Experiment No. 1

ZEBU CALF No. 3281 (weight 129 lb.) and ZEBU CALF No. 2562 (weight 110 lb.)

Inoculated subcutaneously with 50 mg. of six-day-old serum grown culture B.V.28.

Controls. Two rabbits inoculated with 10 mg. of the same culture subcutaneously. One rabbit died 18 days later. Post-mortem examination showed a tuberculous abscess perforating the lumbar muscles into the peritoneal cavity. Liver, spleen and lungs full of small tubercles.

The second rabbit died after 58 days. Post-mortem examination showed acute generalized

tuberculosis. Local abscess. Lungs, spleens, kidney and all glands tuberculous.

Experiment No. 2

ZEBU CALF No. 3281.

Killed after a period of 90 days. Condition fair. Weight same as at time of inoculation, *i. e.*, 129 lbs. Regional gland (right prescapular) enlarged and indurated and on section contained firm, dry, caseous material tending to calcify. A soft purulent abscess in adjacent subcutaneous tissues. Presternal gland enlarged and indurated and on section showed fibro-caseous material tending to calcify. Liver showed numerous very tiny miliary tubercles below the capsule. Hepatic gland slightly enlarged. Lungs normal in consistency but containing numerous very small translucent miliary tubercles.

A guinea pig inoculated with 0.5 c.c. of an emulsion from the prescapular gland died of severe generalized tuberculosis 106 days later.

Sections. *Lung:* Intervalveolar septa generally thickened and congested with occasional circumscribed consolidations composed of fibroblasts and lymphocytic cells disposed in a matrix of structureless hyaline-like material with or without a calcified centre. No giant cells present.

Liver. Very small tubercles—few in number. Epithelioid cells almost entirely replaced by a ring of lymphocytes. Centre of tubercles shows destruction of liver and in some cases complete necrosis, whilst in other cases there was an infiltration with lymphocytes and epithelioid cells. Giant cells frequent.

ZEBU CALF No. 2562.

Killed after a period of 90 days. Increase in weight 34 lb. Condition fairly good. Regional gland (right prescapular) consisted of a large caseo-fibrous tumour-like mass. Presternal gland enlarged and gland substance entirely replaced by dry caseo-fibrous material tending to calcification. Spleen showed a few miliary tubercles scattered throughout the pulp. Liver showed numerous miliary tubercles scattered throughout the organ. Lungs showed a few discrete translucent miliary tubercles.

The impression obtained from the post-mortem examination of these two calves was that the general tendency was one of resolution and healing. There was a distinct tendency to calcification in the gross lesions in the glands whilst the miliary tubercles in the parenchyma of the organs were discrete and firm in consistency, giving the impression of sequestration and resolution.

The pulmonary tubercles in calf No. 3281, described as translucent, appeared to be undergoing a hyaline change.

(1) ZEBU CALF No. 3279

Received 50 mg. subcutaneously of strain B.V. 28 on May 31, 1938. Killed May 20, 1939. Duration of infection: 354 days. Weight at commencement: 132 lb. Weight at conclusion: 284 lb.

Post-mortem. Condition good. Regional gland (right prescapular) about twice normal size and surrounded by a thick fibrous capsule. A tuberculous abscess about the size of a walnut well encapsulated and containing dry caseo-fibrous material is seen at the upper pole of the gland. The rest of the gland is normal with the exception of a small encapsulated abscess about 1 c.m. diameter containing dry calcified tuberculous material. No lesions in the lungs and rest of body normal. This animal has completely recovered from the tuberculous infection and was in perfect health.

One guinea pig injected with 0.5 c.c. of emulsified prescapular gland died of generalized tuberculosis after 189 days.

Sections. *Lung:* The section has all the characteristics of a normal lung. There appears to be a slight thickening of some of the intervalveolar septa, and there are very occasional tiny circumscribed, fibrosed areas involving perhaps six or seven *alveoli*. The fibrous tissue is laid down in a concentric manner and in the centre there may be a microscopic cavity or a small dot of calcified material.

(2) ZEBU CALF No. 3277

Received 50 mg. of strain B.V. 28 on May 31, 1938. Killed January 14, 1939. Duration of infection: 228 days. Weight at commencement: 153 lb. Weight at conclusion: 243 lb.

Post-mortem. Excellent condition and shows every sign of health. Regional gland (right prescapular) about three times normal size and full of creamy yellow pus forming a large tuberculous abscess. Lungs showed a few scattered millet seed-like tubercles—hard and obviously healed.

Spleen normal in size and showed old millet seed hard tubercles showing early calcification. Middle cervical gland firmly encapsulated and containing caseo-calcareous matter.

Mediastinal gland slightly enlarged and indurated. 0.5 c.c. of caseous matter from prescapular gland emulsified and injected subcutaneously into two guinea pigs which died of acute generalized tuberculosis 124 days later.

This animal showed intermittent pyrexia for the first month but gained steadily in weight.

Section. *Lung:* Most of the section shows normal lung tissue. There are occasional areas where the alveoli are filled with a cellular exudate.

composed of epitheloid and lymphocytic cells whilst there are also occasional concentric fibrosed foci involving a few alveoli.

(3) ZEBU CALF No 3275

Received 50 mg. of strain B. V. 28 on May 31, 1938. Killed May 20 1939. Duration of infection: 354 days. Weight at commencement 185 lb. Weight at conclusion: 380 lb.

Post-mortem. Excellent condition and appears in perfect health. The only abnormality seen was the regional gland (right preescapular), which was about twice the normal size—haemorrhagic on section and showing an encapsulated tuberculous abscess about 1 c.m. in diameter and containing fibro-caseous material showing early calcification. Lungs, spleen and all other glands normal.

This animal had obviously recovered from the tuberculous infection and shows almost complete resistance.

It showed intermittent pyrexia for the first three weeks but gained steadily in weight.

Sections. Lung: Lung normal except for very occasional minute circumscribed fibrosed areas involving only one or two alveoli. There are also very occasional areas of circumscribed consolidation consisting of a mass of lymphocytes rather resembling a lymph node. These usually occur near a small bronchiole and occupy three or four alveoli only.

Controls. (1) Rabbit No. 1071: Received 10 mg. of B. V. 28 on May 31, 1938, subcutaneously and died after 92 days of severe generalized tuberculosis. *Post-mortem:* Local abscess. Tuberculosis of lungs and spleen and all glands and raised tubercles on kidneys.

(2) Rabbit No. 1072: Received 10 mg. of B.V. 28 on May 31, 1938, subcutaneously and died after 99 days of severe generalized tuberculosis. *Post-mortem:* Local abscess. Tuberculosis of lungs and spleen and all glands. Raised tubercles on kidneys.

Experiment No. 3

ZEBU CALF No. 3274

Received 50 mg. of strain B.V. 28 on August 27, 1938. Killed May 20, 1939. Duration of infection: 266 days. Weight at commencement: 140 lbs. Weight at conclusion: 168 lbs.

Post-mortem. Fair condition. Regional gland (right preescapular) slightly enlarged and firm and showing a few discrete encapsulated tubercles about the size of pea and tending to calcify. A subcutaneous tuberculous abscess in this region had already drained. Caseo-fibrous lesions showing early calcification were seen in the left preescapular, presteral, mediastinal, axillary (right) hepatic and left precrural glands.

Spleen showed numerous firm encapsulated tubercles 3-8 mm. diameter throughout the organ. Lungs showed many translucent firm miliary tubercles throughout. There were numerous tubercles 3-4 mm. in diameter scattered over the omentum.

The liver showed numerous firm small miliary tubercles below the capsule and throughout the organ. All showed early calcification. This animal had not resisted the infection as well as others but it had gained in weight and the tendency was to complete clinical recovery.

Sections. Lung: This section shows rather more extensive and diffuse areas of consolidation consisting of masses of epitheloid cells in which there is evidence of early fibrosis. There is no giant cell formation. There are several circumscribed and fairly large tubercle-like formations consisting of masses of epitheloid cells through which there are well-marked concentric stands of fibrous tissue and in the centre an irregular area of calcification. Other areas are very tiny circumscribed areas of scar tissue involving only two or three alveoli with or without a tiny centre of calcified material.

Controls. Goat No. 1106 and Goat No. 1107 inoculated with 10 mg. of same strain subcutaneously. Both died 39 days later and on post-mortem showed severe miliary tuberculosis of the lungs with a local lesion in the regional gland—right preescapular.

Experiment No. 4

ZEBU CALF No. 3272

Weight 152 lb. Inoculated subcutaneously with 50 mg. of a six-day-old culture B.V. 34 on December 22, 1939. Killed 91 days later.

Post-mortem. Weight 175 lb., an increase of 23 lb. Excellent condition. Right preescapular gland (regional gland) enlarged, indurated, and full of dry caseo-fibrous material. All other glands normal. Lungs showed a very very scattered miliary tubercles with no peritubercular reaction.

Sections. Lung: A section of a normal lung with very rare tiny areas of concentrically arranged scar tissue involving one or two alveoli and having in the centre a dot of calcified material or a small cavity, or they may be entirely solid.

Liver: Normal except for rare and very small and irregular-shaped localized infiltrations of darkly stained stellate cells. Regeneration of liver tissue appeared to be complete.

Controls. (1) Goat No. 987: Inoculated subcutaneously with 10 mg. of the same culture. Died after 30 days of severe generalized tuberculosis.

(2) Rabbit No. 986: Inoculated subcutaneously with 10 mg. of the same culture. Died after 87 days of severe generalized tuberculosis.

Experiment No. 5

ANKOLE CALF No. 3588

Received 50 mg. T.B. strain B.V. 34 seven-day-old culture on serum subcutaneously on March 14, 1939. Killed *in extremis* April 4, 1939. Duration of infection 21 days.

Post-mortem. Calf very emaciated. Right preescapular (regional lymph) gland enlarged and indurated and lying in a mass of fibro-caseous material. Mediastinal gland enlarged and indurated. Other glands enlarged and indurated. Lungs distended and full of milky tubercles. Liver—numerous milky tubercles. Kidneys—milky tuberculosis. Spleen—milky tuberculosis. This calf showed no resistance to infection and died of acute milky tuberculosis.

ANKOLE CALF No. 3585

Received 50 mg. seven-day-old culture of strain B.V. 34 on serum, subcutaneously on March 14, 1939. Died after 59 days.

Post-mortem. Emaciated. Both lungs distended and full of tubercles about 3 mm. in diameter. Marked hemorrhagic reaction around the tubercles. Spleen swollen and on section showed numerous tubercles. Preescapular, preternal, mediastinal, mesenteric, and hepatic glands all tuberculous. Liver—dark in colour, swollen and covered with numerous tubercles ranging in size from a millet seed up to a diameter of 3.4 mm. These extended throughout the organ.

Controls. (1) *Rabbit No. 1177:* Inoculated intravenously with 0.001 mg. of same culture. Died after 41 days. *Post-mortem:* Severe generalized milky tuberculosis.

(2) *Rabbit No. 1178:* Inoculated subcutaneously with 10 mg. of same culture. Died after 117 days. *Post-mortem:* Severe generalized tuberculosis.

Experiment No. 6

ANKOLE CALF No. 3583

Inoculated with 50 mg. of strain B.V. 34 on March 15, 1939. Weight: 252 lb. Died August 16, 1939. Weight: 250 lb. Duration of infection: 154 days.

Post-mortem. Condition poor. Lungs show numerous milky tubercles. Liver showed milky tubercles. Regional gland enlarged and full of caseous material. Glands generally enlarged and indurated.

ZEBU CALF No. 3582

Inoculated with 50 mg. of strain B.V. 34 on March 15, 1939. Died on October 25, 1939. Duration of infection: 194 days.

Post-mortem. Calf in poor condition. The only sign of tuberculosis was a number of small millet seed tubercles in the spleen and an abscess

in the regional gland. Lungs and liver—normal. This calf was suffering from a severe gastro-enteritis which was the probable cause of death.

Control. *Goat No. 1171:* Inoculated subcutaneously with 10 mg. of the same culture. Died after 41 days of acute milky tuberculosis.

Discussion

The effect of the tubercle bacillus on the tissues depends on the virulence of the particular strain used and on the resistance of the host. Thus the reaction will differ with similar hosts inoculated with different strains and the same standard strain may provoke different reactions in hosts of the same species. Further, it has been observed that the reaction of a susceptible host to a strain of low virulence is similar to that of a resistant host to a fully virulent organism.

In these experiments the strain of tubercle bacillus was of a standard virulence and the object was to determine the degree of resistance of the hosts concerned—Zebu cattle—in view of the observations in Uganda which were recorded in Part I of this paper.

It is generally accepted that a bovine strain of standard virulence will kill an average calf of the European breeds in about 10 weeks when inoculated subcutaneously in a dose of 50 mg. [A. S. Griffith, 1930].

From the results obtained it will be seen that of the eight Zebu calves inoculated all showed a marked degree of resistance, whilst the three Ankole calves showed little resistance and reacted in a similar manner to European breeds. It would seem that in the resistant Zebu calves there was a marked generalized infection which was gradually overcome to a greater or lesser extent, depending on the degree of individual resistance, but in all cases there was sufficient resistance to preserve health, whilst in some an almost completely successful reaction had taken place.

Thus calves killed at 90 days after inoculation showed macroscopic evidence of a fairly severe infection whilst those which were allowed to survive for longer periods up to 354 days improved markedly on the earlier ones and demonstrated a progressively successful and almost complete resistance to a lethal dose of a virulent strain of tubercle bacilli.

The sections examined, although few in number, demonstrate the general histo-pathological process of recovery. Thus in the calves allowed to survive 90 days there was evidence of marked tissue reaction going on in the lungs whilst the lungs of an animal infected for 354 days were quiescent and merely showed a few scars to indicate the battle which it has successfully survived.

The rabbits and goats, as controls, amply demonstrate the virulence of the strains used in each individual inoculation. The above results have been obtained with standard European strains and work is in progress to test out locally isolated virulent bovine strains in a similar manner.

Summary

The results of inoculating subcutaneously eight Zebu calves and three Ankole calves with 50 mg. of a bovine strain of tubercle bacillus of standard virulence are recorded. The Zebu calves showed a marked resistance to tuberculous infection as compared with calves of British breeds, but the Ankole calves, on the other hand, proved equally susceptible.

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A CLINICAL SURVEY OF INTOXICATIONS OF CATTLE BY SUDAN GRASS (*SORGHUM SUDANESE*)

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INTRODUCTION

In the past a good many misapprehensions have existed concerning the toxic properties of sudan grass and its hybrids. It has been stated often that pure sudan grass is not toxic and that mortalities occur because impure seed is used by farmers. Sorghum has always been regarded as highly toxic, and sorghum-sudan grass hybrids are said to inherit the toxic properties of sorghum. Clinical observations made during the years 1931-1938, inclusive, throw a great deal of light on the whole question. The clinical data concerning some thirty-six mortalities have been tabulated and surveyed with the object of furnishing field evidence in support of certain contentions. In presenting this data in tabulated form it is admitted that much quite important clinical material has had to be omitted, but the inclusion of all the relevant observations would make the recorded matter much too unwieldy in form.

During the heavy mortalities of 1931, it became imperative to take advantage of the material offering for investigation, and a great deal of valuable work was conducted at the Glenfield Veterinary Research Station, much of which confirms clinical findings and places some aspects of the matter on a more exact basis.

The object of this article is to record relevant clinical observations. Discussion will be confined to the subject matter given in the accompanying table and will be arranged under appropriate headings.

THE IDENTIFICATION OF PLANTS AND SEEDS

It became obvious in 1931 that allegedly pure sudan grass seed (*Sorghum sudanese*) was capable of producing crops containing plants which differed widely in characteristics. The so-called pure forms consisted of heavily stooling plants bearing a multitude of fine stalks, long narrow leaves and uniform, fine seed. The height of the plants was usually from two to four feet and they had the general appearance of a 'grass'. Coarser forms had fewer stalks, which were much thicker, grew much taller and carried much coarser leaves and irregularly shaped seeds. Sometimes one encountered a crop which looked nearly pure, while in other cases many, or even the majority, of the plants showed all grades of coarseness. It was found that botanical experts had some difficulty in giving a precise opinion upon specimens submitted for determination, and for this reason the name 'sudan grass' as used in the accompanying table means a plant grown from certified sudan grass seed but which may have developed any of the characteristics described above.

It soon became very apparent that no difference in toxicity existed between any of the forms of crops which developed from pure seed. In other words, it was obvious that toxicity did not depend upon hybridization with the coarser sorghum species and that the fine and pure types of sudan grass could be just as toxic as the hybrids. The field observations on this aspect of the matter are supported by analytical data secured by the Veterinary Research Station.

TOXICITY IN RELATION TO STAGE OF GROWTH

It has been stated often that the crop in flower or seed may be fed off with impunity, but clinical observation proves this assertion to be utterly erroneous and dangerously misleading. The clinical observer does not quickly forget the occasions when he arrives at a farm to find a sudan grass paddock of several acres with most of the crop in flower and seed, and a dozen or more dairy cows dead and dying after a quarter of an hour's grazing. One good example of such a crop was No. 29 in the table. It has been argued that it is the immature plants that are toxic, but against this one has been careful to submit leaves from the mature forms for analysis. Moreover, the farmer who risks his cattle on unsound advice is not concerned with any neat distinctions, and if the crop is well grown and in seed then that is good enough for him, but to his undoing.

It will not be denied that immature crops are probably more toxic than mature crops, but it must be stressed that clinical observation indicates that the plant is never safe.

It seems fairly obvious that some disturbance to the even growth rate of the plant is the main factor in rendering it toxic. It has often been stated that a stunted crop is the more dangerous, but such has not been borne out in these observations. How intimately the toxicity factor is bound up with seasonal conditions will be shown in the table and in the next paragraph.

THE EFFECT OF SEASONAL CONDITIONS

Sudan grass mortalities always occur in waves and one can always anticipate when they are likely to occur. Moreover, when one accident occurs it is usually followed by a number of others in the same or widely separated districts; and at the same time sheep mortalities may occur as the result of the ingestion of well-known cyanogenetic plants such as *Cynodon* sp., *Chenopodium carinatum*, and *Euphorbia drummondii*. This widespread occurrence and diversity of mortalities in sheep and cattle as a result of eating cyanogenetic plants is not just chance, but is due to the occurrence of seasonal conditions which favour the production of cyanogenetic glucoside in the plant.

Sudan grass is usually planted in the spring (October) and with sufficient soil moisture or rain a good crop results. This being a winter rainfall district, it is normal for the summers to be intensely hot and dry, and under these conditions the crop does not grow, and even though it be sparse and stunted in the paddock and cattle have access to it, no ill-effects follow. If, however, the crop survives till mid-summer and rain occurs under conditions of high temperatures, the sudan grass grows prolifically and the common practice among

dairy farmers is to put their cows on to the crop for an hour or so each day. Under such a system the crop holds its growth, stools out freely and milk production increases. These are the seasonal conditions which induce toxicity of crops and mortalities in cattle; but yet another factor seems to be necessary and this will be discussed under the next heading.

A glance at the table will show the years when trouble has occurred. They were February-March, 1931, December, 1931, January, 1932, then nothing occurred until the series commencing in December, 1934, and ending in January, 1935. There was thereafter again a period of immunity from trouble till December, 1937, when followed another series of losses which ended the next month.

THE PART PLAYED BY GRASSHOPPER INVASIONS

The seasons already referred to as favouring the good growth of sudan grass are also those which favour the hatching, swarming and invasion of the district with grasshoppers.

These pests advance up the valleys of creeks and rivers and attack anything that is green. Sudan grass they devour in any stage of its growth and remain on it long enough only to eat the crop to the ground, so that viewed from a little distance the paddock appears bare. To do this the swarms may remain from two to ten days and then pass on. During the visitation many of the plants are eaten into the ground and are completely destroyed.

In a showery summer, some rain falls after the crop has been eaten back, warmth continues and the re-growth is very rapid. Meanwhile, owing to a lack of feed and in order to allow the sudan grass an opportunity to recover, the cattle are kept out of the paddock for a period, generally in the region of two weeks. The first grazing of the crop after this spell is the fatal one and usually symptoms are manifest in about ten minutes. In that time sufficient of the crop may be eaten to kill half the herd. A reference to the table will show how almost invariably the grasshoppers have been associated with these mortalities, so that one has come to regard these pests as almost an essential factor. It is considered, however, that their action must be a mechanical one only, and that if some other factor was responsible for cutting the crop in precisely similar circumstances and the same favourable conditions for re-growth occurred the crop would doubtless be just as toxic as if eaten back by grasshoppers. Factors which might occur in practice are mowing and heavy stocking. The broom millet crop referred to in No. 35 of the table is the only one of the series known to have been cut. Cutting right back by grazing seldom occurs, it being the usual practice to allow a limited daily grazing; but when such has occurred, the herd is

usually rotated to another sudan crop on the farm, returning to the original crop when the first has regrown. In these circumstances intoxication and

loss has not occurred, and one wonders therefore if some sort of tolerance to the toxic factor is not developed by the herd.

Details of poisoning of stock grazing on Sudan grass

No.	Date	Crop	Condition	HCN content	Results	Remarks
1	6-3-31	Sudan grass	Six inches high	Strong positive to picrate test	2 cows, 1 severely affected and recovered	No treatment
2	March, 1931	Sudan grass and hybrids	Eaten right down by grasshoppers	(1) Sudan, 0.011% (2) Hybrid, 0.015% (3) Hybrid, 0.008% (undried)	60 cows, 11 deaths, 15 recoveries, Deaths in 25 minutes	No treatment
3	23-2-31	Sudan grass	Eaten bare by grasshoppers two weeks previously. Short re-growth	0.050% (undried)	20 cows, 2 showed symptoms in 10 minutes and recovered	No treatment
4	14-12-31	Sudan grass and hybrids	Eaten bare by grasshoppers. Disaster on re-growth a week later	0.051% (undried)	26 cows. All affected. Symptoms in 5 minutes. First deaths in 10 minutes. 17 dead in 30 minutes. Balance recovered	No treatment
5	2-1-32	Sudan grass and hybrids	Eaten bare by grasshoppers. Mortality on re-growth two weeks later	Sudan, 0.065% Hybrid, 0.066%	11 cows. 1 died in 5 minutes. 1 sick and recovered.	No treatment
6	6-1-32	Sudan grass and hybrids	Eaten bare by grasshoppers. Mortality on re-growth two weeks later	Sudan, 0.029% Hybrid, 0.039%	4 cows. 3 dead in 15 minutes.	No treatment
7	10-1-32	Sudan grass and hybrids	Eaten bare by grasshoppers. Cow died on re-growth three weeks later	Sudan, 0.024% Hybrid, 0.026%	1 cow. Sick in 5 minutes, dead in 15 minutes.	No treatment
8	6-1-32	Sudan grass	Eaten by grasshoppers. Re-growth coming in to head, 12-18 inches high	0.031% 0.028%	5 cows. 1 sick in 10 minutes and died.	No treatment
9	Jan., 1932	Sudan grass	In seed	0.133% 0.068% 0.031% 0.030% 0.014% 0.026% 0.066%	Fed with impunity	These figures are of distinct interest because the cattle were never taken off the Sudan paddocks and in spite of high HCN figures the crops were grazed without accident
10	Jan., 1932	Sudan grass	18 inches. Not eaten by grasshoppers	0.132%	Fed with impunity	Cattle kept continuously on Sudan crops
11	Jan., 1932	Sudan grass	Eaten back by grasshoppers. Re-growth starting to flower	0.026%	Fed with impunity	Cows did not reject the crop after foiled by grasshoppers
12	12-12-31	Sudan grass	Eaten back by grasshoppers and re-growing	Strong positive to picrate test	16 cows. Symptoms in 20 minutes. All affected and 5 died	No treatment
13	23-12-31	Sudan grass	Re-growth after being eaten back by grasshoppers	0.084% (moisture-free)	24 cows. Symptoms in 5 minutes. 3 dead. 12 recovered	The 12 recovered cattle were treated with ether hypodermically and artificial respiration
14	27-12-31	Sudan grass	Two feet and in seed	Strong positive to picrate test	12 cows. 6 sick and all recovered. No symptoms for an hour	No treatment
15	17-1-35	Sudan grass	Sparse and eaten by grasshoppers but re-growing	0.71% (moisture-free)	25 cows. Symptoms in 20 minutes. 13 sick. 6 died	Recoveries treated with ether, atropine and artificial respiration
16	6-12-37	Sudan grass	Eaten bare by grasshoppers. Six inches regrowth when grazed	Strong positive to picrate test	20 cows. All sick, 2 deaths	No treatment
17	10-12-37	<i>Sorghum</i> sp.	Eaten back by grasshoppers, and re-growth grazed five days later	Strong positive to picrate test	6 deaths	No treatment
18	6-12-37	Sudan grass	Eaten back by grasshoppers. Deaths on the re-growth	Strong positive to picrate test	12 cattle. All affected 4 deaths	No treatment
19	4-12-37	Broom millet (<i>Sorghum</i> sp.)	Eaten back by grasshoppers. Death occurred on re-growth	0.167% (moisture-free)	5 cows and a bull died	

Intoxications of Cattle by Sudan Grass
Details of poisoning of stock grazing on sudan grass—contd.

No.	Date	Crop	Condition	HCN Content	Results	Remarks
20	10-12-37	Sudan grass and hybrids	Eaten back by grasshoppers	Strong positive to picrate test	5 cows. All showed symptoms in 10 minutes. No deaths	Treatment first adopted of 3 grammes sod. nitrate, 15 grammes sod., thiosulph., 20 ml. water with astonishing results
21	28-12-37	Sudan grass	Eaten back by grasshoppers. Re-growth one foot to 15 inches high. Not in seed	Strong positive to picrate test	45 cows. 5 became sick and recovered	The 5 sick cows were treated as (20) above
22	24-12-37	Sudan grass	Crop eaten down by grasshoppers and re-growth two feet high, but not in seed	Positive to picrate test	56 cows. 3 dead 5 sick and recovered	The 5 sick cows were treated as for (20) with also artificial respiration
23	5-1-38	Sudan grass and sorghum.	Eaten by grasshoppers and re-growth scanty and up to 12 inches high	Both strong positive to picrate test	4 cows. 3 sick and all recovered	Sick ones relieved by puncturing rumen with trocar and cannula
24	4-1-38	Sudan grass	Eaten back by grasshoppers. Re-growth sparse but vigorous	Strong positive to picrate test	18 cows, of which 13 died. Symptoms in 15 minutes	
25	7-1-38	Sudan grass	Eaten back by grasshoppers. Re-growth sparse but vigorous and out in flower	Strong positive to picrate test	1 cow died at first attempt to graze crop. 3 died at a second attempt two weeks later	
26	10-1-38	Sudan grass	Eaten back by grasshoppers. Re-growth 12 inches and not yet in flower	...	50 cows. 1 died, 8 recovered after treatment	The 8 recoveries were treated as for (20)
27	...	Sudan grass	Eaten down by grasshoppers. Re-growth 12 inches high and in seed	Strong positive to picrate test	60 cows. 6 died in first attempt to graze. At second attempt 10 days later 3 more died and 3 recovered.	The 3 recovered cases were treated as for (20)
28	10-1-38	Sudan grass	Eaten back by grasshoppers. Re-growth 15 inches high and not yet in seed	Strong positive to picrate test	2 cows became affected and both recovered	Treated as in (20)
29	25-1-38	(1) Sudan grass, coarse type.	Eaten back by grasshoppers. Re-growth 2 feet 4 inches high and in seed	0.024%	27 cows. 4 deaths. 3 recovered	5 treated as in (20). 1 died from the treatment owing to 25% increase in the dose.
		(2) Sudan grass, much finer than above.	Not affected by grasshoppers. Also up in seed	0.006%	27 cows grazed with impunity	This crop was fed off and when finished crop adjoining was used.
30	26-1-38	Johnston grass (<i>Sorghum halepense</i>).	Eaten back by grasshoppers. A strip of this perennial had been in the paddock for five years without ill-effects	Strong positive to picrate test	300 sheep. Within an hour 9 died and 3 which sickened recovered	
31	10-2-38	Broom millet	Not known	Strong positive to picrate test	16 cows broke into this crop and 7 died before they could be chased out	
32	15-1-38	Sudan grass	Crop visited by grasshoppers	Strong positive to Picrate test	12 cows. 4 died and 5 recovered without treatment.	
33	10-2-38	Ambrosine (<i>Sorghum</i> sp)	Not known	Strong positive to picrate test	14 cows put on a quarter of an acre and all died	
34	March, 1938	Johnston grass (<i>Sorghum halepense</i>)	A contaminant in another crop	0.060%	45 cows. 11 deaths. 7 recoveries.	No treatment.
35	12-4-38	Broom millet	Crop cut and re-growth grazed three weeks later	Strong positive to picrate test	40 cattle. 2 deaths.	
36	6-5-38	Seacale (<i>Sorghum caffrorum</i>)	Cattle grazed on this crop daily for some weeks	Strong positive to picrate test	45 cows. 11 sick and all recovered	4 recovered without treatment and 7 with treatment

EVIDENCE THAT CATTLE MAY BECOME TOLERANT
TO TOXIC SUDAN GRASS

Inserted in the table is a series of analytical figures referring to several paddocks of sudan grass in which no losses occurred (No. 9). All paddocks were on the same small farm, some were severely damaged by grasshoppers, while others were affected to a less extent. In all, there was sufficient of the crop left to allow the cattle a daily feed right through the grasshopper invasion. Specimens of the plant were taken for analysis from time to time while the cattle were feeding off it. The resulting figures were all extremely high; much higher than those for many of the fatal crops, yet no sickness or losses occurred. At the same time, cattle were being lost under standard conditions on farms almost adjoining (Nos. 5, 6 and 7). Other examples are less striking and are cases in which a few cows of little value or a few head of young stock have been left in a crop right through the period of invasion and re-growth, and have suffered no ill-effects. The case of No. 10 is not comparable, for the crops on that property suffered little or no damage from grasshoppers.

THE EFFECT OF HUNGER

There is both theoretical and practical evidence that hungry cattle, which have eaten a paddock of toxic sudan grass, will succumb more rapidly. Hungry cattle will obviously eat more plant in a given time and therefore will more quickly absorb their toxic quota.

Experience has shown, however, that it is not necessarily safe to attempt grazing off toxic crop with cattle which are not hungry. Such crops must be regarded as valueless for cattle, though they may be used for sheep with a high degree of safety.

SHEEP ON SUDAN GRASS

Sudan grass has been quite a popular summer feed for sheep, especially fattening lambs, and quite often farmers have been advised to turn sheep on to a crop which has proved toxic for cattle and with one small exception no ill-effects have followed. The crop referred to in No. 16 of the table was used to fatten 200 lambs after the accident to the cattle and three of the lambs died, presumably from HCN poisoning. At times when cattle mortalities were occurring, sheep were being grazed on sudan grass under exactly similar conditions and with no harmful effects. The only sheep mortality referred to in the table (No. 30) was in somewhat unusual conditions and on Johnston grass, which is considered to be far more toxic than sudan grass.

SYMPTOMS

The symptoms shown by cattle in HCN poisoning from sudan grass are not very distinctive. An

increased rate of respiration is constant and this may be shown before any other abnormality is noted. A cow first ceases feeding and stands in an agitated, restless manner; the respirations increase and marked distress soon follows. There may be some salivation and odd animals rush about and bellow. Many cases go down quite early and lie prostrate, moaning and breathing rapidly. Death may take place in a few minutes, but some cows will remain down for some hours and then die. Cases that are on the ground for any considerable time usually become affected with hoven.

LATENT PERIOD

The period between the time of access to the crop and the onset of symptoms varies, the variation being due to the amount of crop eaten in the time, and to the concentration of hydrocyanic acid in the crop. Reference to this latent period is made in the table. The shortest observed time is five minutes and it is probable that symptoms were obvious in most cases in ten minutes and the death of the first case would occur shortly afterwards. Reference will be made elsewhere* to the amount of plant that might be expected to be lethal under varying conditions of toxic concentration, but clinically it has been obvious that in some cases a pound of the plant was sufficient to cause death.

TREATMENT

A consideration of the data in the table will show that mortality may be heavy or absent, in the latter case the animals recovering completely. Very many cows recover without any treatment. Many cases die much too quickly for treatment to be applied, but the treatment of animals which survive for half an hour or more may be the means of saving their lives. Generally the farmer himself has neither the knowledge nor the means to do anything of benefit. The sudden collapse of a number of his cattle and the early death of some of them leads him in desperate haste either to the telephone or to his neighbour, and on at least three occasions the farmer has been known to cut off the animals' tails to make them bleed.

For a long time it has been regarded by some that the liberal sub-cutaneous injections of ether has a very beneficial effect, the drug being used to give a quick stimulant effect. Ether was tried on one occasion only (No. 13). The twelve treated animals were given 20 mls of ether about fifteen minutes after they went down, followed by another 20 mls. The farmer credited the injection with saving the cattle, but there was no evidence that it was of benefit. Artificial respiration has been

* Hind Marsh, W. L. Poisoning of Cattle by sudan grass and other Gynogenetic Plants. *Austr. Vet. J.* 16, 219

employed on prostrate animals whose treating had become slow and shallow, probably with benefit. In animals showing tympany, the use of the trocar and cannula to relieve intra-abdominal pressure on the thorax is undoubtedly an essential precaution.

In 1937 a line of treatment was adopted which gave results of immediate and very obvious benefit. The formula adopted was based on the suggestion of Bunyee (1935) and consists of the hypodermic injection of the following solution :

Sodium nitrate	3 gm.
Sodium thiosulphate	15 gm.
Water	20 mls

This injection has been given to a number of cattle which appeared to be on the point of death ; some of them have responded immediately and have got up within a few minutes, and all of the cattle treated have recovered. There has been sufficient evidence to support the opinion that this treatment is specific. There is evidence, too, that cattle will not tolerate a dose much in excess of that mentioned.

SUMMARY

1. An account of a number of serious losses from sudan grass poisoning has been given and is sup-

ported by a tabulated statement of some principal data.

2. There is evidence that pure sudan grass may be just as toxic as are its hybrids with sorghum.

3. The seasonal conditions which predispose to intoxications are those in which rain falls during the heat of the summer, inducing optimum growth of the plant.

4. Sudan grass which has been eaten to the ground by grasshoppers and which is re-growing rapidly after rain has proved, in almost all cases, to be at the dangerous stage.

5. A few mortalities on other sorghum species such as ambergene, Johnston grass and broom millet are also recorded.

6. Particulars are given of what appears to be a specific line of treatment, especially if adopted along with symptomatic treatment.

7. In conclusion it can be postulated that none of the sorghum species can be considered safe to feed off to cattle at any stage of their growth following the conditions which have been described.

REFERENCE

Bunyee, H. (1935). *J. Amer. Vet. med. Ass.* 86, 666

INQUEST ON A CURRICULUM

(Reprinted from *The Lancet* August 30, 1941)

KNOWLEDGE of medicine is handed from seniors to beginners according to the Hippocratic rule, and perhaps in no other profession is learning so generously and directly shared. But medicine stands alone in that the leaders and teachers live and practice in a different world from the great body of the profession. It is an old cry that the aim of the medical curriculum should be to turn out good general practitioners: how do we set about it? From the moment of registration the student is taught by specialists, most of whom have no experience of general practice. At the end of the course, and of a few resident appointments, he is well—even brilliantly—equipped to start specializing. He has a sound general background of all that is rare in medicine and surgery, but he has learned to think of common diseases as humdrum. He can discuss points of technique in all the specialities; but he has had no chance to treat the minor ailments, because these do not reach the hospital clinic. In the one direction—obstetrics—where specialization would not come amiss he is under experienced. He has usually delivered about twenty normal maternity cases

(without analgesia) and has watched the R.O.G. dealing with abnormals; in general practice normal cases will usually fall to the midwife while he is summoned, often late, to the abnormal case. He has had no instruction whatever in certification or the management of a panel practice; he is usually shaky on prescribing; he may or may not be a competent anaesthetist; he thinks the business of making the patient comfortable is work for the nurse; preventive medicine seems to him to be the province of the public health service; his ideas about the social and industrial responsibilities of medicine are vague and general; and he does not even realize that to sign a patient off as 'fit for work' is an act of faith unless he is clear what strains that work applies. He begins his life's work as a fumbler, lacking half the tools of his trade; and he learns skill at the expense of his patients. Every general practitioner remembers his first week in the surgery, the confusion of mind produced by the rich variety of certificates, the growing sense of dis-appointments as 'just another rheumatism' came in, the guess-work which supplemented his ignorance when the patient asked for

the details of some nursing procedure. To the patient he was a young chap not quite up to it; to himself he was a young chap far beyond it: and both were true—he could have dealt competently with an obscure or alarming condition, he was only at a loss with chronic and minor illness. But these are to be his life's work; if he is ill-informed about them at the outset he is at a disadvantage; and if he is conditioned to find them boring he is handicapped indeed.

There is another way in which the medical student's training fails him: he has worked where it was always possible to give the patient something like the best in the way of examination, investigation, treatment, feeding and environment. He now finds that he is continually driven to make shifts and compromises which seem, at first, to stamp him as a hypocrite. He discovers not only that he cannot do the best for his patients but that he has not even been taught to do the best he can. Of course he learns. He picks up a hint from a colleague here, a trick from the district nurse there; he supplements inadequate diets with costly vitamins because he must not prescribe food; he gives tonics where he would like to give blankets, cod-liver oil where he should order a sojourn in the country. He begins to realize that he can only patch up where, given proper equipment he could restore. Here the fault lies not wholly in his training; it is right that he should know the best in treatment and learn to apply it. The defect is in a medical service still unequipped in many directions to command the essentials of healthy living. And it is not our duty, any more than it is the duty of the crowner's quest, to give praise or blame, to bring any of the agents to judgment, but rather to find out by patient inquiry how the prewar curriculum came to be the poor frustrated thing it was, and to share in the glorious task denied to the coroner of infusing new life into the old framework.

INQUEST OF A CURRICULUM

There are grave difficulties about training a man to be a doctor, and it would be all too easy in the present emergency to emphasize the utilitarian side at the expense of his fundamental education. Witness the reaction against experimental method, contemptuously called *Kaninchenmedizin* in Central Europe. This danger is naturally more vivid to the minds of teachers than of students. Teachers see clearly that if practice is not based on a sound appreciation of scientific method and though it will deteriorate rapidly when the student leaves his medical school. They seek to provide a better grounding; the suggestion reaching us from many deans is that physiology should be an integral part of the whole curriculum and that, in the teaching of anatomy, structure should be continually related

to function. At Sheffield anatomy and physiology are taught throughout the five years of training and an examination in these subjects has been introduced after the second M.B., a year before the finals. Some feel that the time adjustment between preclinical and clinical work and between the start of clinical work and finals is wrong. From St. Thomas's comes the view that time is wasted by the student in cramming the minutiae of anatomy; and that the premedical subjects might well be disposed of by the attainment of an acceptable standard in matriculation or school certificate. The student could then go to a central school of anatomy—there might be two for the London area—for 9 to 12 months. He would concentrate on the essentials of anatomy and enough relevant physiology, taught by the anatomists, to explain function. At the end of the course he would take an examination in which attainment of honours standard could qualify for the primary fellowship. He would then join his clinical medical school which would be equipped with a department of physiology. For a year he would study normal physiology, physiological chemistry and clinical methods, including the use of stethoscope, auroscope and ophthalmoscope, and would learn to interpret radiograms. He would also learn the technique of urine testing, blood-examination and other laboratory tricks. He would take another examination at the end of a year before passing on to clinical work and the study of morbid physiology which is medicine. He would not be eligible to sit for finals until he had completed 36 months of clinical work, but to keep him nettled up there might be sessional examinations at the medical school. At present students are able to sit for part of the finals within 30 months of the second M.B., and thus the average student is cramming for a part of the final within 24 months of beginning clinical work. This suggested curriculum, admirable as it is, lays emphasis chiefly, on groundwork and is not directed towards the special needs of general practice.

Students and general practitioners have different suggestions to make. On the whole students see more of general practice than do their teachers; many of them are the children of general practitioners and many hear the opinions of recently qualified friends. Moreover that concern for social conditions which springs from imaginative appreciation of a neighbour's hardships—socialism in the basic sense—flourishes among the young. If we are not willing to put the world right in our early twenties we are poor citizens. The British Medical Students Association (p. 259) ask that the student should receive better training in preventive medicine and gain more experience of chronic and minor

ailments and the simpler gynæcological conditions; that he should have a wider knowledge of public health, of national health, insurance, of compensation and rehabilitation. They see the general practitioner, in short, as part of the social welfare organization of the country and would have him competent to undertake that duty. Nor are they oblivious of the groundwork on which such training must rest. They ask for greater integration in the curriculum, and for orientation courses which would bring the various subjects into proper relationship at the outset. One subject in which an orientation course would be particularly valuable is anatomy; a teacher suggests that the whole body might be dissected rapidly before the class, by an expert, before students are allowed to begin on a part. All who remember the bewilderment of their first month in the dissecting-room will agree with him. Some of the detail might be cut out and he suggests that anatomy should be taught on younger subjects, preferably beginning with the foetus, so that students may see the organs at various stages of development, not only in the degeneration or atrophy of old age.

Not unnaturally, the most practical suggestions for improvement of the curriculum come from general practitioners themselves. They would like the student to have free access to the patients in the chronic wards of infirmaries, to see more minor infectious illnesses like tonsillitis, and more minor injuries like sprains. They would like him to have personal experience of dispensing, not of writing 'rep. mist.' and leaving the rest to the dispenser; to be confident of giving a safe anaesthetic; to know some practical psychology and more about mental disease and defect; and to realize that there is no mystery about children's diseases or infant feeding. They think he should know more about the trifling ills of women—such as vaginal discharge, dysmenorrhœa and menopausal symptoms—and that he should have some instruction about sterility and contraception. He should be more adept at recognizing the early stages of disease, especially of cancer, and of rheumatic disorders likely to lead to crippling. Above all they would have him a competent obstetrician. Some would like six months post-graduate work in midwifery to be compulsory for the general practitioner. Others consider that instead of spending a year on general surgery and month on midwifery, the periods might well be reversed, or at least more wisely distributed. Students spend hours in the theatre watching major surgical and gynæcological procedures which, as general practitioners, they will never perform. They enjoy it, naturally; most of us like to assist, in the French sense, while a skilled craftsman does his job, whether it be

painting a picture, building a ship, or opening an abdomen. But watching major operations is a waste of time in a crowded curriculum and a scrutiny of the student's timetable would doubtless reveal other extravagances; one doctor reminds us of the time spent loitering in the front hall, awaiting dilatory chiefs. Time saved from these and other pursuits might be spent with a good firm of general practitioners. Some doctors think the grounding would be adequate if the student attended at such a practice once or twice a week for a month or two; some think he should be resident with the firm for periods ranging from a fortnight to six months; others think this experience could be post-graduate; but none doubts that the person to train another in general practice is the general practitioner. This return to the apprenticeship system, they feel, is overdue. They do not suggest that it can in any way replace formal clinical teaching; only that it should supplement it, occupying time which can be spared from less essential studies. All are emphatic about the value of resident appointments and some would like such post-graduate experience to be compulsory for a man about to enter general practice.

The foregoing opinions—whether from teachers, students or practising doctors—are home and Empire produce; they are what we can learn from each other. But the experience of other countries is also instructive, especially when it forms a comment, spoken or unspoken, on our own practice. The accounts of medical education in various parts of the world set out on page 260 will repay study. We have something to learn, for example, from the Spanish system of selection, by which the best opportunities go to the best students; from the high standard of general education expected of students in France; from the student discussion groups of Norway; from the German tradition of experience in several universities; from the keenness, diligence, and team spirit found in Russian clinics; and from the American principle that medicine deals not with the disease alone but with its effects on the patient, the patient's family and the community. Russia, travellers tell us, finds us backward in our treatment of women; Spain thinks the golden age of English medicine is over, Germans are politely astonished when they find an English institution efficiently run, and Americans have to stop and think when they are asked to name the advantages of English medical education. It is time to make changes. Remembering the old maxim for examination candidates—'do the easy ones first'—let us begin by introducing experience of general practice into the curriculum, and a higher standard of obstetric training among those deciding to become family doctors.

The composition and properties of goats milk

G. M. TROUT (1941). *Bull. Mich. agric. Exp. Sta.*, 23, 254-64

FOURTY samples of bulk milk from individual goats and from herds on analysis gave an average fat percentage of 3.91 ± 0.75 and total solids 13.60 ± 1.04 . The fat percentage of the milk varied widely between the breeds. Fat was determined by Babcock method, total solids by the Majoumder method and the S. N. F. was calculated from the values obtained for fat and total solids. Nubian milk was rich in fat (4.41), Steagon milk slightly less (4.18) and Toggenberg milk least (3.54). The ash content of 17 samples of goat's milk showed an average 0.923 per cent, with a range from 0.872 to 0.972 percent. The ash content was slightly higher than that of cows milk. The ash content of breed milk varied slightly with the fat. Specific gravity determinations of 17 samples gave a mean of 1.0337, with a range from 1.032 to 1.035. The titrable acidity of 39 samples of fresh milk brought to the laboratory packed in ice well below 50°F ., ranged from 0.12 to 0.275 per cent with an average of 0.190 per cent. This is contrary to the opinion that goat's milk is alkaline in reaction when compared with cow's milk. The pH of the goat's milk (19 samples) ranged from 6.33 to 6.52 which is a lower figure when compared to cow's milk. The average flavour score of 49 samples was 21.75 which is to be recognized as good flavour. Of the above 19 samples scored 23 and above having no flavour criticism. Of the off flavours present, those of 'feed' were noted in 10 samples. [G. P. A.]

A simple differential medium for mastitis testing

J. G. DAVIS (1941). *Dairy Indust.* 6, 38

THIS new medium, Brom-cresol purple chalk agar, suggested for mastitis work has the following composition:-

Peptone (1)	3 gm.
Lactose (2)	5 "
Yeastol (3)	3 "
Leuco (4)	3 "
Precipitated chalk (5)	10 "
Agar	15 "
Separated milk	10 ml.
Brom-cresol purple (0.04 per cent aq. sol.)	50 ml.
Tap water	1000 ml.

Peptone, leuco, yeastol and agar are dissolved in water by heating and filtered hot. The pH of the medium is adjusted to 6.8 with Bromthymol blue using a Hellige comparator. Lactose, milk, chalk and Brom-cresol purple solution are added and filled in sterile test-tubes with constant shaking and then sterilized at 15 lb. pressure by momentary autoclaving. The remarkable feature of this medium is that the acid-forming colonies dissolve the chalk-forming zones. In the freshly drawn samples of milk from the cows suspected to be suffering from mastitis, when plated on this medium, the zones formed are generally due to *Streptococcus agalactiae*, the causative organism of mastitis. In normal aged samples the acid-producing types will form zones by dissolving the chalk.

N. B.

- (1) Any peptone may be used.
- (2) Dextrose may be substituted to detect those organisms fermenting dextrose but not lactose.
- (3) The yeast autolyzates on the market are roughly of equal growth promoting value.
- (4) Any reliable meat extract may be used.
- (5) The precipitated chalk should be sterilized in dry condition for three days at 200°C . [E. V. S.]

The control of parasitism in sheep with phenothiazine in a salt lick.

J. W. BRITTON R. F. MILLER and H. S. CAMERON. *Cornell Vet.* 32, 400-406

OBSERVATIONS were made on Ladino clover pastures in California, on which approximately 250,000 lambs are annually fed. The stomach worms, *Ostertagia* and *Trichostrongylus*, constitute a source of great menace in these lambs. In the past, anthelmintics such as tetrachlorethylene and a mixture of copper and nicotine sulphates were used. These indications are not, however, very effective, for, as Gordon has estimated, about 12 per cent of the lambs fail to respond to the drugs, because of the failure of the oesophageal reflex to operate in time to direct the drug into the abomasum. Furthermore, the labour involved is very costly, and the starving of lambs overnight and for several hours after treatment retards the rate of growth. Mass administration of anthelmintics has not so far been attended with success due to the toxicity of the agents employed. The authors used a mixture of phenothiazine and half-ground salt in the proportion of 1:15 and made it accessible to lambs as the only salt lick. The results of the experiments showed that the mass administration of this mixture was nontoxic and very effective when given over a period of 78 days. The total dose necessary for the effect was approximately half the minimum single therapeutic dose. This combined with the ease of administration and the elimination of the preliminary starvation period reduces the cost of the treatment considerably. Repeated small doses administered in this manner maintain a continual small concentration of the drug in the alimentary canal. This at first might retard or inhibit the development of eggs, later inhibit the egg production, and finally prove lethal to partially paralyzed worms. When a single therapeutic dose is administered, roughly half of the drug is absorbed and excreted in the urine. In the case of repeated small doses little, if any, is absorbed from the intestinal tract and this explains why only half the total amount of the therapeutic dose of the drug is required in this case. The lambs on the medicated salt mixture consumed on an average slightly over 22 grams of phenothiazine per head during the period of 78 days. There were no toxic symptoms or staining of the wool and the faecal cultures indicated that the lambs were free from parasites. The method appears to offer great possibilities [G. D. B.]

Immunization against bovine trypanosomiasis

H. E. HORNEY (1941). *Trans. R. Soc. trop. Med. Hyg.* 35, 163-76

THE author has critically reviewed the work of Schilling (1936) on the immunization of African bovines against trypanosomiasis. Schilling's contentions were as follows: (1) young animals possess a considerable resistance against trypanosomes transmissible by tsetse flies, and (2) this resistance is enhanced to full immunity by minimal infection or by preventive vaccine treatment. The author had the opportunity to follow in detail the fate of the experimental herd of Schilling, and he found that when this herd was transferred to well-known tsetse belt the animals developed the disease. It was observed that of all the animals vaccinated or otherwise treated by Schilling in 1934 only one out of 38 was alive at the close of the experiment (21st January 1941). Schilling continued his observations from June 1934 to July 1938 and he came to the following conclusions which were based on the evidence of survival at the end of this period:—three out of twelve control calves, four out of nine vaccinated

calves, and eight out of seventeen calves treated with minimal infective doses. He believed that these animals were immune in the way that the most large African game animals within tsetse-fly belts are. Subsequent history of these surviving animals, however, as indicated above was different, and the author has consequently come to the conclusion that "no method of immunization which will enable East African domestic stock to thrive in tsetse-fly belts has yet been devised". [F. N. R.]

The value of dogfish meal as a protein supplement. M. REHAN, J. S. CARVER, R. W. HARRISON, and W. S. HAMM (1942). *Bull. agric. Exp. Sta.*, State Coll. of Washington, 416, 1-24.

VARIOUS experiments were carried out to determine the effects of different methods of processing on the gross value of meals made from dogfish.

The data presented indicate that the commercial dogfish meals available in Washington were of a low nutritive value as judged by growth experiments carried out on chicks. Other experiments were carried out to determine the relative values of wet and dry methods of processing the meals. Wet process meals were prepared as follows: the fish were ground, cooked with live steam at atmospheric pressure, pressed to remove oil and water, and dried in a steam vacuum drier. The dry process meals, were prepared by drying the fish in a steam-jacketed vacuum drier and removing the oil by pressing the dried product in a hydraulic press.

The wet processed meals gave as good results per unit of protein as ordinary fish meals and very much better results than commercial or laboratory dry process catfish meal.

The data also suggest that the quality of fish meal proteins deteriorates fairly rapidly after manufacture and that it is very probable that the fish meals as used under commercial conditions, are much poorer in value than meals at the time of manufacture.

An initial trial with materials treated with formaldehyde prior to processing suggested that such treatment may be harmful to the resulting product. However, later trials demonstrated that the harmful effect obtained in the initial trial could not be attributed to formaldehyde treatment.

The essential difference between the wet and dry process treatments of the fish meal used is that a large amount of water soluble material is removed during the wet-process. The hypothesis is put forward that the wet-process meals may have lost some substances which is either toxic to the chicks or which adversely affects the protein.

A sample of commercial (dry-process) dogfish meal, which was re-processed by the wet-process gave considerably better growth results in chicks than the original type of meal.

From the results of this experiment in conjunction with those of earlier work the authors conclude that, though ureic acid is not utilized by the chick, it is not harmful to the feeding value of a feed except that it affects the determination of the protein content.

The average protein (N/6.25) of the commercial (dry-process) dogfish meal was 77.3 per cent, and that of the wet-process dogfish meal was 67.5 per cent. It is suggested that unadulterated dogfish meals of exceptionally high protein content (N/6.25) especially with a high ureic content, are likely to be dry-process meals. [A. J. M.]

Various oils and fats as substitutes for butter fat in the ration of young calves. T. W. GULLICKSON, F. C. FOUNTAINE and J. B. FITCH (1942). *J. Dairy Sci.*, 25, 117

THE comparative feeding value of vegetable oils like coconut, peanut, corn, cottonseed and soybean oils, and animal fats, such as butterfat, lard and tallow, for young dairy calves was measured by the rate of gain in weight and by observing the physical appearance and general well-being of the animals. Calves were put under experiment at the age of one to two weeks. Fats or oils, mixed at the rate of 3.5 lb. to every 96.5 lb. skim milk and homogenized to give a product containing 3.5 per cent of the fat or oil, were fed to the respective groups of animals, with the following low fat content concentrate mixture-dry beef pulp, 200 lb.; dry skim milk, 50 lb.; gluten milk, 50 lb.; cod liver oil U. S. P., 35 to 35 cc. and some alfalfa hay. Two control groups were run simultaneously, (1) entirely on a fat-poor ration, skim milk (0.01-0.02 per cent fat) along with the following concentrate mixture-ground molasses beet pulp, 100 lb. dry skim milk, 50 lb.; starch, 50 lb. and cerelese, 25 lb.; and (2) on whole milk not homogenized. The periods ranged from a few days to about six months. The average daily intakes of nutrients per 100 lb. live weight of calves fed various fats and oils were 1.62 lb. for the whole milk, 1.82 lb. for the butter oil, 1.57 lb. for the low fat, 1.83 lb. for the lard, 2.0 lb. for the tallow, 1.71 lb. for the coconut oil, 1.96 lb. for the peanut oil, 1.71 lb. for the corn oil, 1.59 lb. for the cottonseed oil, and 1.37 lb. for the soybean oil groups. The average daily gain in the weight of calves of the respective groups were 1.43 lb., 1.22 lb., 1.07 lb., 1.17 lb., 1.24 lb., 0.96 lb., 0.80 lb., 0.40 lb., 0.31 lb., and 0.32 lb.

The calves fed butter fat excelled those in all other fat or oil groups in physical condition, general well-being without any complaint of sour and in average gain in weight. Corn-oil-cotton seed oil and soybean-oil fed groups were the least satisfactory, and the animals were most severely affected with indigestion or sour among the other groups, and some of them died.

Another significant difference noted between the groups was the greater deposition of fat in the carcasses of the milk-fat-fed calves. [B. C. R. S.]

Enzymatic production of bacterial polysaccharides.

M. STACEY (1942). *Nature*, 149, 639

SEVERAL investigators have reported the synthesis of Polysaccharide by enzymic preparations obtained from various species of spore-forming bacilli. The author also describes some of his experiments, in this line, which are of considerable importance in the study of the mechanism of symbiosis of yeast and bacteria.

He found that *Leuconostoc mesenteroides*, grown in symbiotic association with *Saccharomyces cerevisiae*, forms mucoid dextran more rapidly than when grown alone. The production of this dextran, at one stage, is attributed to 'exocellular enzymatic action'. The enzyme system is, on the other hand, suggested to be sharply stimulated by a factor elaborated *in situ* by the living yeast cells.

The purified dextran [2%] + 180° in water contained N 0.5 per cent and gave only glucose on acid hydrolysis, [N. B. D.]

The value of the rapid whole blood-stained Antigen Agglutination test in the eradication of pullorum disease. H. P. GORDON and G. C. BRAUDER (1942). *Vet. Rec.*, 54, 275-9.

THE comparative value of the rapid whole-blood agglutination test, using stained antigen, and the routine

serum tube test for the diagnosis of *S. pullorum* infection was investigated under field conditions. A total of 1750 tests were carried out on three infected farms whereby an agreement of 98.34 per cent was established between the two tests. Twenty-nine birds gave conflicting results, but on bacteriological examination *S. pullorum* was recovered from 15 out of 26 birds thus tested. Actually out of 228 reactors, including those from which the casual organism was isolated the rapid whole-blood test and the serum tube test showed an error of 6.1 and 0.4 per cent respectively. In similar trials carried out on 1519 birds, from which *S. pullorum* could not be isolated, the serum tube test may have condemned 0.5 per cent and the rapid test 0.2 per cent.

Better results were achieved in subsequent test made at the laboratory when an agreement of 99.31 per cent was established. The authors have since used the rapid test on three other fairly heavily infected farms and concluded that this method, as a preliminary, has the advantage of eliminating a large percentage of infected birds. [J. A. L.]

Studies with equine streptococci. 3. Vaccination against strangles. P. M. BAZELEY (1942). *Aust. Vet. J.*, 18, 141-55

The view has been expressed after consulting the literature, that there is no satisfactory immunizing agent against the disease Strangles. The author using a cautiously heat killed vaccine showed that a significant immunity could be established in mice. In the present paper he has recorded his results on horses using five types of vaccines. They are classified as follows:—

- Vaccine 1. Formalin-killed young-culture organisms.
- Vaccine 2. Formalinized 30-hour culture filtrate.
- Vaccine 3. Formalin-killed whole young culture.
- Vaccine 4. Formalinized 24-hour whole culture.
- Vaccine 5. Cautiously heat-killed young-culture organisms.

One strain of *Streptococcus equi*—572 was used throughout for the preparation of these vaccines, and proved 100 per cent satisfactory in the type of growth and yields obtained. All the vaccines with the exception of the filtrate (2) and 24 hours whole culture (4) were stored at a temperature of 0° C. and 8° C. as their immunizing properties were seen to deteriorate when kept at room temperature. When put to field use, the vaccines were naturally exposed to an abnormally high temperature, and deterioration was noticed in Vaccine 3, the keeping quality of Vaccine 5, was, however, in no way greatly lessened on account of this exposure, due probably to more exact and better conditions of manufacture. In two field trials the vaccines were injected into groups of horses to determine whether an immunity against Strangles could be set up. The first test on 1749 horses with vaccines 1, 2, 3, and 4 proved inconclusive, but in favour of a cautiously killed young culture vaccine. In a second and more comprehensive test using exclusively a heat killed young culture vaccine (No. 5) the product showed definite immunizing powers, and appears to be the biologic of choice for the prevention of this disease. [D. A. M.]

The effect of large scale active immunization against anthrax. MAX STERN, J. NICOL and M. C. LAMBRICHTS, *J. S. A. V. M. A.*, 13, 53-63

With a view to assess the effect of large scale active immunization of cattle against anthrax on the subsequent incidence of the disease the authors have collected relevant data from several localities representing areas where active immunization had been practised to different degrees of intensity. The Transkei was a notoriously badly affected locality. Data are presented (a) for

eleven districts in this area where 'block' inoculation of cattle against anthrax was practised from 1928, (b) for 15 other districts in the same area where 'block' inoculation had not become completely enforced until 1934; and (c) for the district of Mt. Currie where 'block' inoculation of cattle had never been practised; (d) for the province of Natal where 'block' inoculation was carried over parts of the territory, while in other parts inoculation had been carried out only if a case of anthrax occurred or had occurred in previous years; and (e) for the rest of South Africa where control was not very effective, about $\frac{1}{3}$ to $\frac{1}{2}$ of the cattle population only being inoculated each year mostly at the option of the farmers.

The data collected represent figures for each year from 1925 to 1941 and comprise of the total number of animals in the locality, the number inoculated, the number of outbreaks reported and the number of deaths from anthrax. As an index to the accuracy of reports of cattle mortality the authors use the number of smears sent up for examination, which has convinced them that the returns reflect the actual state of affairs as regards the Transkei and Natal from the year 1935.

A critical examination of the data collected has led the authors to make the following observations:

"There is much less anthrax in well-controlled and well-inoculated areas than in those where inoculation,—although on a considerable scale—is more haphazard. In the Transkei, once a heavily infected part, annual inoculation of all cattle is associated with a low incidence of anthrax. There is evidence that anthrax decreased more rapidly in the districts where 'block' inoculation was first carried out". Anthrax was now only rarely seen in the Transkei, many districts having been clean for several years. The decrease of anthrax in Natal, although notable, was not so marked as in the Transkei probably because 'block' inoculation of the entire cattle population was not practised. As regards the rest of South Africa this position was much more worse corresponding with the more haphazard nature of the inoculations. [V. R. R.]

Sulfaguandine feeding as a control measure for cecal coccidiosis of Chickens. MARION M. FARR and REX W. ALLEN (1942). *J. Amer. vet. med. Ass.*, 100, 47-51

The authors have demonstrated the prophylactic action of sulfaguandine against cecal coccidiosis in very young chicks (12 to 16 days old). When fed with 1 or 2 per cent sulfaguandine mash for three days before and nine days after inoculation with *Eimeria tenella* (43,000 oocysts) birds showed no symptoms, lesions or oocidial forms. This drug has no curative effect since birds receiving three or five per cent medicated mash daily at the first appearance of blood in the droppings showed no significant benefit. It was observed that birds which were protected from an initial infection by sulfaguandine were highly susceptible to a reinoculation of 93,000 oocysts 29 days later. The average gain in weight showed by the inoculated controls was found to be less than that of the birds receiving prophylactic doses of sulfaguandine and 'greater than that of the birds receiving medicated mash as a curative agent.' [H. N. R.]

Observations on the control of bursate nematode by the chemical treatment of manure. T. W. M. CAMERON, and I. W. FARRELL (1939). *Vol. Jub. Yoshida*, 2, 319-29

BURSATE round worms affect both human beings and domestic animals. Attempts to destroy parasitic stages

in the host have had very encouraging results in man, horses and dogs; much less so in ruminants. The application of mass treatments of man and animals is not, however without its disadvantages: financial as well as administrative and consequently comparatively little has been done. There remains, therefore the treatment of faecal material before it is used as manure. This may be physical, chemical or both. The physical treatment involves natural or artificial heat as a killing agent. Natural heat may be generated in a well-packed manure pile or in an insulated manure box. Artificial heat involves a specially constructed manure box.

Comparatively little has been done to find chemical methods of destroying the free-living stages of the nematodes. It is a problem which presents many difficulties probably the greatest of which is the fact that the eggs and larvae are always in close contact with faeces, soil or grass, and many chemicals which might be used are partially or completely counteracted by contact with organic matter. Nevertheless chemical control of all free-living stages does appear to have considerable practical possibilities. It offers opportunity of using faecal material as manure and even of enriching the manure and it does not necessitate special equipment.

The joint authors record their experiences with ninety-eight chemicals while dealing with *Sclerosotoma* of horses.

1. Some chemicals such as flowers of sulphur destroy only the fungi without affecting the eggs or the larva of worms.

2. Some chemicals, such as ferrous sulphide, ferric oxide, ground limestone, rock phosphate, basic slag, dorr, white holocore, and pyrothrum powders are inert.

3. Some chlorides and sulphates, sodium and potassium hydroxide and potassium permanganate, occasionally cause exsheathment. These chemicals, in slightly greater proportions, generally also cause the death of the larva.

4. A large number of chemicals mixed with faeces in certain proportions allow a considerable number of eggs to hatch and the larva to reach the infective stage and then comparatively rapidly kill the larva. Such chemicals are quicklime, cupric, ferric and ferrous sulphates, zinc sulphate and zinc sulphide, the chlorides of copper, iron and zinc, cupric nitrate, sodium fluosilicate and oxyquinoline sulphate. Other chemicals which cause delayed death are nicotine sulphate, trisodium phosphate, sulphate and chloride of manganese, sodium and magnesium borate strong cresol, phenol and calcium hypochloride.

5. Larvae having thick sheath and the egg in the centre of the hump of faeces may be protected from the action of chemicals.

6. Applied dry chemicals which are deliquescent have more prospect of being of practical value, for the moisture which they attract may also attract the larva. With very lethal chemicals, such as iodine salts, it has been found that very considerably less chemical is necessary to produce sterilization with a very weak rather than with a very strong solution.

Some chemicals such as chloropicrin, calcium cyanide, naphthalene, O' and para' dichlorobenzene can be used as gases.

The cost of the chemical has also to be taken into account. Urine has more or less all the advantages. Another class of chemicals are the artificial fertilizers. Aniline and pyridine are lethal both to fly larvae and sclerosotoma larvae. The advantage of such a dual sterilizer is obvious from the economic point of view.

At the end of the article is given a list of chemicals used, with the percentage necessary to cause sterilization and the strength of the solution to be used. [G. D. B.]

Studies on the chemical composition of the blood of dairy cattle III. The normal concentration of inorganic phosphorus in the whole blood of dairy cattle and factors affecting it. LANDINGHAM, H. O. HENDERSON, and G. A. BOWLING (1942). *J. Dairy Sci.*, 25, 529.

The authors have studied the normal values of the concentration of inorganic phosphorus in more than 600 samples of whole blood taken from 59 dairy cattle—cows and heifers, during the periods of growth, gestation, and lactation at different seasons of the year and with and without exposure to sunlight. The Fiske and Subbarow method for inorganic phosphorus was followed for analysis.

They observed no indication of any difference in the inorganic phosphorus content of the blood as a result of the ration supplying liberal quantities of phosphorus.

In the case of growing heifers calves, age was found to be the most important factor affecting the concentration of inorganic phosphorus in the blood; which showed an increase with age up to about the sixth or seventh month and then a gradual decline as the animals grew older. The maximum value, 7.15 mg. per 100 ml. whole blood, was recorded during the seventh month.

With lactating cows, a decrease in the blood inorganic phosphorus was noted with an increase in the number of lactations up to about the third or fourth lactations. During the period of first gestation a lower concentration of inorganic phosphorus (4.81 mg. per 100 ml. whole blood) was observed in the blood of heifers than in that (5.68 mg. per 100 ml. whole blood) of non-pregnant heifers of the same age. The inorganic phosphorus value showed practically no change throughout the first lactation period, but dropped readily near the end of the second gestation period and during the first three months of the lactation, thereafter the value increased to same level as during the first lactation. The value showed again decrease in the latter part of the third gestation period and for the first two or three months of the third lactation. After the third lactation no significant change in blood composition was observed.

The data obtained for growing heifers from the age of one month to the time of first calving did not indicate any seasonal effect upon the inorganic phosphorus content of the blood. The fact that the animals were confined to the barn continuously or permitted to run outside in favourable weather, also made no difference. However, there was a strong tendency for dairy cows after the first lactation to show a lower concentration of inorganic phosphorus in the blood during the winter and early spring than during the summer and early fall. [S. A. M.]

Vitamin A deficiencies in ruminants. H. SCHMIDT. (1942). *Amer. J. vet. Res.*, 2, 373

The author studied the effect of the Vitamin A deficiency in food on cattle, sheep and goat. He described the symptoms, depletion period and the effect of supplementing the feed with the sources of Vitamin A and carotene. The minimum requirement of this constituent in cattle, sheep and goat for maintenance was also worked out.

One group of cattle was fed on a vitamin A deficient ration consisting of cottonseed meal, cottonseed hulls and white grain. The control group received alfalfa hay in addition to the deficient diet. After 120 days on this feed the experimental animals stopped growing and started to lose weight. They also showed night-blindness.

discharge from the eyes and nose, in some cases ulceration of the cornea, rapid respiration during the warmer part of the day and frequent convulsions. Four of the experimental animals were given different doses of cod liver oil and they made a rapid recovery. Three other animals were given the same doses of cod liver oil in which the vitamin A was destroyed and the animals showed no improvement. At this stage the feed was supplemented with alfalfa hay and pure crystalline carotene in maize oil. The animals made a rapid recovery. The animals without vitamin A supplement died on the 179th day of experiment. Observations showed that night-blindness was the first noticeable symptom in the development of vitamin A deficiency syndrome, and the author used it as a guide in determining the depletion of vitamin A body reserve. It was ascertained that the optimum requirement of carotene for cattle was 2,500–3,000 γ per 100 lb. body weight, and this would prevent the animal from developing night-blindness.

Further experiments were arranged to determine the vitamin A requirement of the sheep and angora goats. The picture of vitamin A deficiency in sheep and goats was different from those of cattle. Loss of appetite lasting for several weeks, loss of weight with an unhealthy appearance, night-blindness and nasal discharge could be noted. Opacity of the cornea swelling of the limbs and convulsions were not a constant feature. The depletion time varied according to the exact seasonal time that they were put on the experiment. It was noted that the depletion time of the goats born around the first of March and placed on the deficiency diet in latter part of November was longer than those animals born about the same time and placed on experiment in the middle of February. This indicated that young animals grazing during their first summer acquire a definite body store of vitamin A. The survival period in sheep and goats on vitamin A free diet varied from 226–455 days and 160–312 days respectively. When night-blindness was used as a yardstick, the determination of the depletion period in sheep and goat was much more difficult than it was in cattle. Also there were no other clinical indications to determine accurately the depletion period. It was pointed out that 750 γ carotene per 100 lb. of live weight might be sufficient under ordinary circumstances but in the case of stress might be too low. It was also pointed out that in the vitamin A deficient animals urinary urobilinogen developed from 112–343 days.

The author described the sources of vitamin A under natural condition. Green plants contained abundance of carotene but as soon as the plant died, be it when it was cut for hay or from other causes, the destruction of the carotene began and proceeded at a rapid rate especially in presence of light.

Rapid drying at high temperature of freshly cut green alfalfa hay preserved much of the carotene.

The author analysed the liver of the young born and found vitamin reserve. He pointed out that they must receive carotene or vitamin soon after birth. [D. N. M.]

Post-mortem changes in New York dressed poultry at 35°F. G. F. SPEWART, BELLE LOWE and MARY MOOR (1941). *U. S. Egg and Poultry Mag.*, 47, 542

Most of the poultry sold in the large consuming markets of the United States of America are New York dressed (bled and plucked but not drawn). During the course of marketing they are held at 30–40°F. for varying intervals, sometimes for several days, before being eviscerated. Consequently there is likely to be variations in flavour and aroma as well as tenderness of the carcass. This investigation was undertaken to determine how long

young chickens (boilers and fryers) should be held at 35°F. to permit the passing of *rigor mortis* and bring about the optimum degree of tenderness, as well as to study the kind and degree of deteriorative changes which occur in New York dressed poultry held at the above temperature.

White Leghorn cockerels weighing 2.3 lb. after being killed, bled and plucked were placed in a room at 35±1°F. After various intervals of time carcasses were removed, eviscerated, ecked and studied. Cooking, palatability and shearing tests were carried out with the meat of these birds.

The results of the study showed that New York dressed birds hung in air at 35°F. began to undergo deteriorative changes within 8 hours after killing; the first of which was the appearance of a bile stain on the liver. Off-odours in the kidney area became clearly noticeable after 24 hours. These gradually spread and intensified finally reaching the wing joint in four days whereafter there seemed to be no further increase. From the flavour scores of the cooked meat it was evident that deteriorative changes set in almost at once as seen in the meat of the thigh and breast muscles and became plainly noticeable in 48 hours but progressed less rapidly in the latter. *Rigor mortis* was found to set in during cooking when birds were held less than 4 hours and often remained even after cooking but never occurred after eight hours holding. Tenderness in poultry meat increased rapidly with the passing of *rigor*. The loss of shearing force in the cooked breast muscle seemed to coincide exactly with the increase in the judges score of tenderness. There seemed to be little increase in tenderness after 24 hours at 35°F. (The term 'shearing force' is used to denote the force necessary to cut off a portion from the whole, in this case the reference is to meat. [T. S. K.]

The effect of high-temperature short-time forewarming of milk upon the heat stability of its evaporated product. B. H. WEBB and R. W. BELL (1942). *J. Dairy Sci.*, 25, 301

The effect of high heat treatment or 'high-temperature' forewarming of milk upon the heat stability of evaporated milk made from it has been studied. Milk standardized to a fat, solids-not-fat ratio of 1:2.20, was forewarmed (by forcing it through 0.18 inch I.D. stainless steel tubular heaters) over a range of temperatures from 101°C. to 165°C. in 4 seconds, held for 25 seconds and cooled to a temperature less than 38°C. in 4 seconds. The forewarmed milk was then concentrated under 28 to 29 inches of vacuum to less than one half of its initial weight, quickly heated to 80°C., homogenized at once under 2500 pounds per square inch pressure, cooled and standardized with water to 26 per cent total solids (18 per cent solids content in the case of skim milk). As skim milk did not always produce the same type of heat stability curve as whole milk, most of the experiments were conducted with whole milk. Control samples were forewarmed (by heating with thorough agitation in a steam jacketed kettle) to 95°C. and held for 10 minutes. Heat stability determinations were made by heating the samples of concentrated milk in small cans in a pilot sterilizer (sterilization temperature 115°C. for whole milk and 120°C. for skim milk) until coagulation was observed. When stabilizing salts were used, 1 ml. of standard salt (phosphate or calcium) solution was added to 130 ml. of milk.

Heat stability of the test samples was generally about two and occasionally as much as six times greater than that of the control samples. The degree of stability brought about was not the same in various samples of milk and the average values for the stability of whole

milks forewarmed to 95°C. and to 140°C. were 45 and 86 milks respectively. Milks of excessive acidity coagulated when subjected to high temperature forewarming, but normal milks of good quality withstood temperatures of 150°C. to 160°C. without coagulating. Undesirable changes in colour and flavour and an increase in acid intensity were noticed when forewarming temperatures above 140°C. were used.

It was observed that milks, falling within the stability range of 25 to 40 minutes, possessed a commercially acceptable body. Milks below this range showed a slight grain or were of excessive viscosity, and those with a stability greater than 40 minutes were thin at the end of an 18 minutes sterilization period. The data indicate that the high forewarming temperature required to produce an evaporated milk of a certain desired viscosity may be within limits 2°C. for one milk or within limits as wide as 60°C. for another milk. The optimum temperature depends upon the nature of the stability curve for each milk and hence its estimation presents some difficulties, although it usually falls between 120°C. and 140°C. when a coming up time of 4 seconds and a holding time of 25 seconds are used. When no clue is available the safest procedure would be to forewarm the milk to 130–145°C. for 25 seconds.

In the case of mildly aged milks, forewarming to the proper high temperature was an effective measure of stabilizing the concentrated milk toward sterilization. Use of the optimum high forewarming temperatures brought about, in the milks tested, a greater increase in heat stability in the evaporated milk than could be attained by the addition of the optimum quantity of stabilizing salts to a normally forewarmed milk. The heat stability and body of evaporated milks could be controlled without the use of stabilizing salts by steriliz-

ing the proper blend of the normally forewarmed and high temperature forewarmed milks and stable concentrate mixture suitable for sterilization could be obtained by high temperature forewarming some 25 to 50 per cent of the milk received in the condensory. [C. P. A.]

Spirit blue agar : A medium for the detection of Lipolytic organisms. STARR. M. P. 1941
Science, 93 (2414), 333

A sensitive differential medium which could not harm appreciably the growth of the organisms and which could detect fat splitting organisms is given. Thirty gm. of agar, 10 gm. of tryptone and 5 gm. of yeast extract are dissolved in approximately 900 ml. of distilled water by autoclaving for several minutes. After complete solution, 25 ml. of 20 per cent cotton seed oil emulsion [100 ml. of fresh cotton seed oil (wesson) 10 gm. of finely ground gum arabic and 400 ml. of warm distilled water and 50 ml. of previously filtered 0.3 per cent alcoholic solution of spirit blue (National Aniline) are added. The mixture is made up to one litre with distilled water] and the medium sterilized by autoclaving for 15 minutes at 15 lb. pressure. Either pour plates or streak plates are recommended. The medium should be stored in the cold to prevent oxidation. Refrigerated sterile plates of spirit blue agar keeps for over 3 months. Colonies of lipolytic organisms are recognized by the development of a permanent deep blue colour beneath the surrounding colony. While examining the growth characteristics and lipolytic activity of about 200 species of bacteria, yeast and molds, no inhibition of growth or lipolysis was noted. Total counts in dairy products and the number of lipolytic organisms in the same could be determined with this medium. [E. V. S.]

ORIGINAL ARTICLES

PATHOLOGY AND PATHOGENESIS OF EXPERIMENTAL FLUOROSIS IN CATTLE

I. BONE LESIONS IN CATTLE FED A DIET ADEQUATE IN CALCIUM AND LOW IN PHOSPHORUS

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(With Plates XVI-XIX)

From biochemical studies on materials from cases of 'osteomalacia' in Hyderabad [Mahajan, 1934-35], it can now be suggested that fluorine is mainly concerned in producing the affection [Majumdar, Ray and Sen 1943]. Clinical and epizootiological findings of Vishwanathan [1934-35] and Ramiah [1939-40] point to the same aetiology in 'rheumatic arthritis' of cattle in Madras.

As regards the pathology of these affections, little information is available, except that clinically and in gross osseous changes, the condition resembles osteomalacia. It was necessary, therefore, to investigate these cases from a pathological standpoint. As regards osseous changes, it is generally agreed [Rohoin, 1937; Cohrs, 1941] that these are greatly influenced by such factors as age, dosage and nature of fluorine compounds, the duration of ingestion, and the animal's prevailing dietetic condition. Before studying materials from spontaneous cases, it was, therefore, decided to examine materials from experimentally-induced cases in which the factors concerned could be adequately controlled. In this paper the pathological findings are described of bones from fully mature hill bulls which had been fed with 3.4 mg. fluorine per kgm. body weight. The animals examined belonged to the 'high fluorine' group of bulls fed by Majumdar *et al* [1934] and an account of feeding of these animals and the dose of fluorine administered can be consulted from a recent publication by these authors.

The following is the clinical protocol of animals of the group.

Hill bull No. 167

Four years and six months, NaF ingestion for about nine months.

Clinical. Gradual loss of weight, general emaciation, hide-bound appearance, skin denuded of hair in patches, bowels irregular, belly

tucked up, haemorrhage from nose, conjunctival membranes anaemic, prolonged decubitus and difficulty in rising, exostoses present on the left metacarpus and left metatarsus.

Blood picture. Rbc.2.6, Wbc.12.5, Hb. 3.6; Lymph 72 per cent, Neutro. 28 per cent, Eosino. Baso. and Mono. 0 per cent.

Hill bull No. 180

Four years and six months, NaF ingestion for about 10 months.

Clinical. Extreme emaciation, soury coat, bed sores on the skin, eyes sunken, lachrymation and excoriation of skin below the inner canthi of eyes, upward curvature of columna, appetite impaired, periodic diarrhoea, hoofs cracked and elongated, and the left hind leg often kept raised from the ground due to fracture of the thigh bone. Right metacarpus showed several nodular growths, left metacarpus showed a flattened exostosis, left metatarsus stouter than the right one.

Blood picture. Rbc. 8.5, Wbc. 12.5, Hb. 8.2, Lympho. 59 per cent, Neutro. 38.5 and Mono. Eosino. and Baso. 0 per cent, and Mono. 2.5 per cent.

Hill bull No. 402

Seven years, NaF ingestion for about 10 months.

Clinical. General loss of condition and weight, hide-bound appearance, loss of appetite, occasional diarrhoea, stiffness of gait, eyes sunken, visible mucosae anaemic, hoofs elongated and cracked. Both the metacarpal bones appeared thicker than normal due to the presence of extensive exostoses occupying more or less the entire anterior surfaces of the bones; right metatarsus had a nodular exostosis about the middle of the shaft and palpable periosteal thickenings on the anterior surface of both ends of the bone.

Blood picture. Rbc. 6.1, Wbc. 5.0, Hb. 9.2, Lympho, 57.5 per cent, Neutro, 42.0 per cent

Eosino. and Baso. 0 per cent and Mono. 0.5 per cent.

Hill bull No. 89

Five years and eight months, feed basal diet alone for nine months.

Clinical. The animal showed no signs of abnormality and no exostoses on any of the long bones.

Blood picture. Rbc. 6.5, Wbc. 8.5, Hb. 11.9, Lympho. 53.8 per cent, Neutro. 30.5 per cent, Eosino. 10.2 per cent, Baso. 0.25 per cent, and Mono. 5.25 per cent.

GROSS PATHOLOGY

The osseous changes under this heading may be considered as common to all animals of the 'High Fluorine Group'. After maceration, all bones appeared whiter than normal, and the exostoses porous on close examination. None of the bones could be cut with a knife, but some, when tested with a saw yielded more easily to a depth corresponding to the thickness of the exostoses. The periosteum over the exostoses appeared to be thickened, closely adherent to the bone and markedly hyperaemic. The marrow was gelatinous and contained reddened patches and dilated capillaries, this feature being more marked in the metaphyses. On longitudinal section of the femur, the compacta revealed an irregular thickness and isolated areas (Plate XVI, fig. 1) of eroded bone. Sections of bone across the exostoses revealed the latter as sharply defined areas, whiter in colour than normal compacta, from which it was clearly demarcated by a line (Plate XVI, fig. 2) and could often be detached during sawing. Generally, the cut surface of the compacta appears smooth, but in areas showing active periosteal growth, the cut surfaces reveal 'islands' of slightly hyperaemic soft tissue, presumably residual masses of periosteum entrapped due to their inability to keep pace with the growing exostoses.

The metaphyses were composed of a smaller number of loose and thin trabeculae enclosing wide spaces containing rather little hyperaemic marrow. The epiphyseal cartilages were wavy in outline and irregular in width. Long bones without palpable exostoses during life revealed thickened subperiosteal tissue which had been transformed into a white granular layer, representing the early stages of an exostosis.

On longitudinal section of the costal epiphysis the cartilaginous junction appeared as an irregular line with tongues of cartilage projecting into the subchondral spongiosa.

Pieces of bone, 2-3 mm. thick, were fixed in 5 per cent neutral formal-saline, decalcified in 5 per cent nitric acid in 60 per cent alcohol followed by washing in 70 per cent alcohol. The tissues were then passed through chloroform to paraffin, according to the technique given by Kolin [1942]. In some cases exostoses and costal epiphyses were examined without decalcification by the method of Thomas, Clark and Schulz [1940], followed by staining by V. Kossa's method or its modification by Moolman [1941].

For histological work the lesions selected were those on the metatarsal and metacarpal bones, the epiphysis and diaphysis of the rib and the distal extremity of the femur. A description more or less common to all the three fluorosis cases is given.

Metacarpus and metatarsus

Cross sections at the level of the exostosis usually revealed three well-defined zones (Plate XVII fig. 1), the innermost corresponding to the old compacta, the middle to the exostosis and the outermost to the periosteum. Microscopically, the exostosis was found to be composed of a mesh-work of trabeculae arising from the outer limit of the old compacta. The portion of exostosis, adjacent to the old compacta, was more compact in appearance, and the trabeculae for the most part better calcified with well-developed osteocytes and only thin osteoid borders. The part of the exostosis towards the periphery, on the other hand, looked more spongy, the trabeculae being arranged more irregularly with wider spaces between them and consisting of poorly calcified axes and wider uncalcified osteoid tissues on either side. The faulty nature and the poor degree of calcification of the trabeculae in this part were revealed by V. Kossa's method as irregular black-stained masses of calcium scattered through the matrix (Plate XVIII, fig. 1). These seldom coalesced to form one continuous black colour as in normal bone. The osteoid tissues were lined by a single layer of osteoblasts, and similar cells were found on the surface of the calcifying osteoid tissues, although the latter also contained osteocytes. The residual masses of periosteum in the growing exostosis showed ossification (Plate XVII, fig. 2) by metaplasia. This was apparently brought about by the formation of numerous acidophilic masses which later coalesced to form trabeculae lined with osteoblasts arising from the periosteal

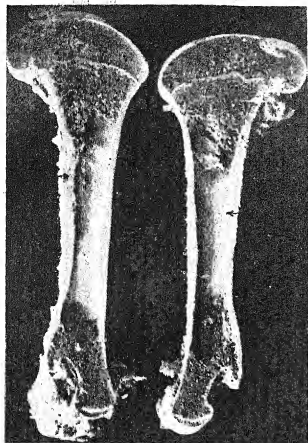


FIG. 1. Case No. 180—Longitudinal section of metatarsus. The bone shows a marked exostosis on one side occupying its whole length, outside the original compacta and demarcated from it by means of a line.



FIG. 2. Case No. 167—Longitudinal section of femur showing irregular thickness and loose texture of compacta. Metaphyseal spongiosa shows resorption.



FIG. 3. Case No. 180—Longitudinal section of the costal epiphysis showing the presence of numerous osteoblasts. Condensation of collagenous fibrils and the formation of new trabeculae by metaplasia. Note the irregular disposition



FIG. 4. Case No. 180—Longitudinal section of the costal epiphysis showing two trabeculae with an apposition of syncytial masses around each. Note osteoblasts and osteocytes, and a syncytial bud arising from the main mass.



FIG. 1. Case No. 167—Cross section of the metacarpus showing the periosteum, the exostosis and a portion of the diaphysis $\times 400$.



FIG. 2. Case No. 180—Cross section of exostosis showing an "island" of periosteum undergoing ossification by metaplasia. Note acidophilic masses and numerous osteoblasts in this region $\times 100$.



FIG. 3. Case No. 167—Cross section of the metacarpus showing a superabundance of young haversian canals in the diaphysis $\times 100$.



FIG. 4. Case No. 167—Longitudinal section of the costal epiphysis showing defective calcification of the trabeculae. Note the osteoid tissue. Van Kossa's stain $\times 300$.

tissue. By the same process of metaplasia, the periosteum at its outer limit was transformed into bone. The intertrabecular spaces contained osteoblasts and other cells of the periosteum. In the region where the periosteum still persisted, the subperiosteal tissue contained numerous osteoblasts, indicating periosseal activity for the formation of new bone.

Histologically, the architecture of the old compacta showed an abnormal disposition of young haversian canals. The formation of these structures at one part of a section was found to have proceeded at a high speed, so that they were closely crowded (Plate XVII, fig. 3), while at other parts no such changes were discernible. Newly formed haversian canals were composed entirely of osteoid tissue (Plate XVIII, fig. 3), which showed poor or no signs of lamellation and irregular calcification. Certain areas in the diaphysis, comprising the outer limit of the old compacta, showed numerous cement lines indicating active periosteal apposition of bone in which the lamellae were poorly calcified and the interlamellar spaces traversed by Volkman's canals. In the course of these canals young haversian spaces were found to be developing, and the previous lamellar arrangement giving place to a haversian system of lamellae formation. This seemed to have arisen by osteoclastic resorption of the existing lamellae, subsequent deposition of osteoid tissues and finally canalization of this tissue. A potential haversian canal, appearing in the osteoid tissue thus formed, contained a conglomeration of osteoblasts, and the same cells were scattered on the osteoid tissue (Plate XVIII, fig. 3), indicating its osteoblastic origin. Haversian canals of similar nature and origin were found in the earlier formed layers of the exostosis, thus tending to obliterate the line of demarcation between the newly-formed bone and the original compacta.

Costal epiphysis

Lesions hitherto undescribed in cattle were found in the rib epiphysis involving the epiphyseal cartilage and the sub-chondral spongiosa. The proliferating zone of cells in the epiphyseal cartilage was absent. The zone of preliminary calcification, which in the normal rib (Plate XVIII, fig. 2) constitutes a sort of grid-like arrangement, had disappeared giving place to a small number of thin and atrophied trabeculae (Plate XVIII, fig. 4), masses of collagenous fibrils interspersed with osteoblasts and a few recently formed trabeculae (Plate XVI, fig. 3). Apparently these had resulted from extensive

resorption of the original trabeculae by osteoclasts or osteoblasts, a subsequent development of collagenous fibrils and finally the formation therefrom of young trabeculae. The collagenous tissue from which the young trabeculae arose, were being transformed into small and irregular matrices (Plate XVI, fig. 3) resulting from condensation of the fibrils into homogenous masses acidophilic in character. These fused together to form trabeculae in which osteoblasts of the original tissue still predominated. The trabeculae thus formed were poorly calcified and the osteoblasts had acquired lacunar spaces and were being transformed into osteocytes. These trabeculae were often irregularly disposed and their osteoid borders while not abnormal in width, unlike those of normally calcified trabeculae, were irregularly demarcated from the calcified zone. V. Kossa's method revealed the axial parts of the trabeculae as irregularly calcified zones (Plate XVII, fig. 4), having rugged borders against the osteoid tissue. The osteoid contained few osteoblasts on the surface and a single layer of similar cells on the free borders.

Lower down in the metaphyseal spongiosa were found trabeculae which, although poorly and irregularly calcified, possessed no definite osteoid borders. Instead, each of them was closely apposed to a syncytium of osteoblasts, some of which were enclosed in lacunar spaces. These tissues appeared to have proliferated, due to the osteogenic property of the osteoblasts, as 'buds' arising from the main syncytial mass (Plate XVI, fig. 4), were being transformed into bone by a direct process of ossification. The intertrabecular spaces contained practically no marrow cells, the chief elements of the marrow being the reticulum and the osteoblasts.

Costal diaphysis

Examination showed a considerable resorption in the cortex and medullary spongiosa. The former although normal in width, was technically transformed into a structure composed of widely separated trabeculae (Plate XIX, fig. 1), having osteoid borders lined by single layer of osteoblasts. The medullary spongiosa was composed of a few unconnected slender trabeculae, lined by osteoid tissues of normal width. Osteoclastic resorption of the trabeculae was also going on in this region of the bone, and the intertrabecular spaces, bounded by resorbed trabeculae, contained disintegrating and a few intact osteoclasts, numerous osteoblasts mixed with collagenous fibrils and osseous debris from resorbed trabeculae.

Distal epiphysis of femur

The normal disposition of trabeculae immediately below the epiphyseal line had disappeared, giving place to irregularly disposed trabeculae of normal structure. These trabeculae apparently were of recent formation since they showed slight clarification (Plate XIX, fig. 8). Some of the more recently-formed trabeculae showed accumulations of osteoblasts at the surface, indicating their osteoblastic origin. The usual process of breakdown and rebuilding of bone had taken place through the activities of osteoclasts and osteoblasts respectively; as evidence of the former phenomenon, the osteoclasts presented a picture of 'dissecting resorption' (Plate XIX, fig. 2) of trabeculae. The intertrabecular spaces were occupied as a rule by net-works of reticulum and osteoblasts, but those immediately below the epiphyseal cartilage contained dilated capillaries and collagenous fibrils, the latter presumably arising from osteoblasts since these cells showed fibrillar projections at either end and contained elongated nuclei. The collagenous fibrils at places showed areas of condensation crowded with osteoblasts, some of which could be seen developing lucunar spaces.

PATHOGENESIS

Chronic fluorine intoxication in animals is a generalized chachectic condition, as the absorbed fluorine compounds affect various organs of the body. In attempting to explain the pathogenesis of the disease, attention has been directed especially to the mineral metabolism, to the relation between fluorine and parathyroid and thyroid, and finally to vitamins, particularly vitamin C. Further, the type of lesion depends on such factors as dose and nature of fluorine compounds ingested, duration of ingestion and the prevailing dietetic condition. On the basis of certain biochemical data and histological findings in affected bones, an attempt may be made to explain certain features of the pathogenesis.

As far as the symptoms and the gross osseous changes are concerned, chronic fluorine intoxication may be looked upon as an osteodystrophia, but the question arises whether, as with certain classical osteodystrophias, the osseous changes in fluorosis also become manifest as a result of disturbed calcium and phosphorus metabolism. Before attempting a complete answer to this question the effects of a high dose of NaF on the calcium and phosphorus contents of blood and bones of the experimental animals may be examined.

TABLE I

Calcium and phosphorus in blood and thigh bones of animals given high dose of NaF (Majumdar *et al* 1943).

Hill Bull No.	Bone (gm. per 100 gm. of ash)		Blood Ca (mg. per 100 c.c. of serum) and P (mg. per 100 c.c. of blood) after 7 months' fluorine feeding	
			Ca	P
167	41.5	17.3	9.9	3.7
180	40.0	17.3	9.6	5.4
402	39.5	17.4	8.6	5.4
Control 89	41.0	17.3	10.4*	4.73*

*Average

The figures in Table I clearly indicate that the high fluorine intake did not lower the calcium and phosphorus values of the blood and bone appreciably. Metabolism studies on these animals by Majumdar *et al* [1943] showed slightly negative Ca and P values. This feature taken in conjunction with the slight lowering of blood calcium might suggest that the pathogenesis of fluorine intoxication is, to some extent, connected with a fall in blood calcium. Against this is the fact that by feeding extra calcium as phosphate and carbonate to another group of experimental animals, which were getting the same quantity of fluorine, symptoms and lesions of fluorine intoxication were not prevented. The negative Ca and P balance observed by Majumdar *et al* may, therefore, have been due to a generalized toxic effect of NaF interfering with the animals' appetites and so leading to lowered intake of Ca and P. Evidently fluorine has no direct effect on the metabolism of these minerals, and its effects on bone can hardly be explained solely on the basis of negative Ca balance.

Histological examination of certain parts of the skeleton, particularly the spongiosa, reveals excessive resorption of trabeculae, indicating a considerable withdrawal of Ca and P. This would suggest a rise in output of Ca and P, but in view of only a slight increase which has already been accounted for, one might agree with Roholm [1937] that the actual explanation of the phenomenon lies not in the negative Ca balance, but in the primary calciprivate mechanism of fluorine affecting the usual calcium depots of the bone. The question therefore, arises as to the fate of calcium withdrawn from these skeletal depots, and this can be explained on the assumption that fluorine



FIG. 1. Case No. 167—Cross section of the exostosis showing a poor degree of calcification as revealed by irregular staining of the trabeculae. Van Kossa's stain $\times 160$.

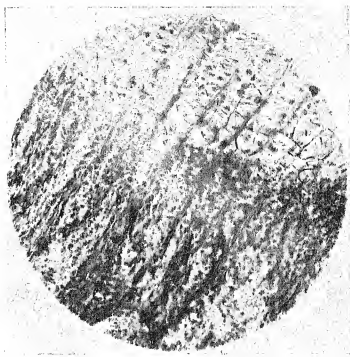


FIG. 2. Case No. 89—Longitudinal section of the costal epiphysis of a normal rib. Note parallel disposition of sub-chondral trabeculae $\times 160$.



FIG. 3. Case No. 402—Cross section of the metacarpus showing two young developing haversian canals. Note an abundance of osteoblasts in the developing lumen and the osteoid tissue $\times 300$.



FIG. 4. Case No. 180—Longitudinal section of the costal epiphysis. The section shows (1) complete disappearance of the zone of preliminary calcification and (2) considerable atrophy of the metaphyseal trabeculae. Van Kossa's stain $\times 160$.



FIG. 1. Case No. 167—Longitudinal section of the costal diaphysis showing widely separated trabeculae in resorbed cortex. Note the trabeculae lined with osteoid borders and the osteoblasts $\times 160$.

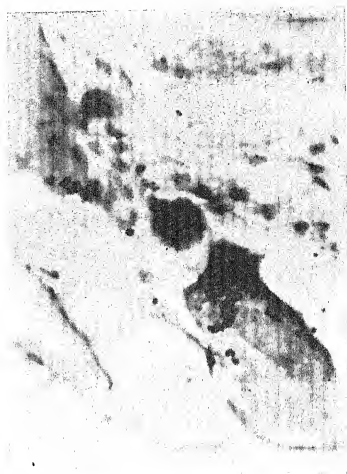


FIG. 2. Case No. 167—Longitudinal section of the distal epiphysis of femur showing "dissecting resorption" of a trabecula. Note the presence of an osteoclast in the centre of a resorbed trabecula $\times 300$.



FIG. 3. Case No. 402—Longitudinal section of the distal epiphysis of femur showing a complete disappearance of the normally disposed trabeculae. Note poor calcification of trabeculae in this region $\times 160$.

apart from its calcioprive property, exercises a special tissue effect leading to the production of hyperplastic changes. These are as follows:

1. Formation of loose osseous tissue between the periosteum and compacta of the long bones which appears as a whitish deposit, or more prominently as an exostosis;

2. Increased functional activity of bone as revealed by the excessive formation of young haversian canals, composed mainly of osteoid tissue, in the diaphyses of long bones;

3. Development of collagenous fibrils and the formation of new trabeculae therefrom in the costal epiphyses, and the presence of new and irregularly laid trabeculae in the epiphyses of other long bones;

4. An excessive proliferation of endosteal cells (osteoblasts), giving rise to young haversian canals in the diaphyses and syncytial masses of osteoblasts in close apposition with the young trabeculae in the spongiosa.

In view of these observations it seems probable that fluorine also stimulates bone formation through osteoblasts in the usual way. The curious but the obvious double reaction of fluorine on the osseous tissue may be explained on the assumption that the calcium mobilized from the spongiosa by its calcioprive effect is utilized for the formation of new bony tissue under the influence of its osteogenic property.

DISCUSSION

The main point for discussion is whether pathologically the condition described in this paper stimulates any known mineral deficiency disease of the bones of cattle. Histologically, we have observed pronounced osteoporosis in certain mobilizable areas of bone, and as according to Theller [1934] osteoporosis in cattle represents not a disease in itself, but a stage in the development of rickets or osteomalacia, question arises to what extent this condition resembles osteomalacia.

Early European investigators [Bartolucci, 1912; Cristiani, 1925; Askanazy, 1930; Hupka and Götze, 1931] have diagnosed bone changes due to fluorine ingestion in cattle as osteomalacia without regard to the following points:—(a) that in fluorine intoxication animals suffer from pronounced cachexia due to toxic effects on parenchymatous organs, (b) that the exostosis in fluorosis appears not only as a nodular growth, but also as a continuous sheet of secondary bone around the primary cortex giving the bone a more or less uniformly thickened appearance, (c) that the long bones show

no deformities and are not oftened and (d) that fractures usually occur at sites of exostosis.

Theller regards osteomalacia of cattle as aphosphorosis, a view which negatives the identity of chronic fluorosis with osteomalacia, unless it can be shown, as in the case of osteomalacia, that under all conditions of dosage, age, diet, duration of ingestion and the nature of fluorine compound ingested, the blood sera of cattle have lowered values for inorganic P. The results of blood analyses and balance studies in respect of Ca and P in fluorine-intoxicated animals are variable. These variable results [Greenwood *et al*, 1933-34; Bester *et al*, 1936; Du Toit *et al*, 1937; Phillips *et al*, 1934; and Ramiah, 1940-41] may be due to varied experimental conditions.

In the present investigation some of these factors were adequately controlled. The experimental animals, all mature hill bulls received a diet adequate in calcium and low in its phosphorus contents, and were fed NaF for 9 to 10 months at the level of 3 to 4 mg. per kgm. body weight. There was no lowering in the phosphorus value of blood of these animals, [Majumdar *et al*, 1943], clearly indicating thereby that the low phosphorus content of the diet did not bring about any symptom of aphosphorosis during the short experimental period.

Microscopically, osteomalacia and rickets are characterized by a superabundance of osteoid tissues. In fluorosis, however, although some osteoid tissue is present, its distribution is irregular and it may be absent from parts of the skeleton where its presence in an excessive amount would generally be expected. This the trabeculae composing the exostoses, the spongiosa of the costal metaphyses and the diaphyses and the metaphyses of long bones are not particularly rich in osteoid tissue, and the small amount of osteoid in the diaphysis of long bones only occurs as remnants of uncalcified osseous matrix around haversian canals undergoing centrifugal calcification. The occasional presence of osteoid in certain easily mobilizable areas of the bone may be attributed to direct deprivation of minerals consequent on decreased appetite.

The important features of fluorosis in these experimental cases which distinguish it from osteomalacia are (1) the interlacing network of collagenous fibrils and numerous osteoblasts in the spongiosa of the limb bones and ribs, (2) the formation of new and irregular trabeculae from the collagenous fibrils and osteoblasts, (3) the absence of distinct osteoid borders around the newly-formed trabeculae, which are in fact

scarcely demarcated from the surrounding cellulofabril masses from which they arise, and (4) the tendency for the formation of syncytial masses of osteoblasts in apposition with the newly-formed trabeculae. The whole picture therefore represents a stage in the development of osteosclerosis, which is a characteristic feature of chronic fluorosis in man and rat. The over-production of young haversian tubes in the diaphyses of long bones, seeming to arise from osteoblasts, is also related to endosteal activity, and here again the same feature of osteosclerosis may be said to predominate.

SUMMARY

1. The osseous changes, resulting from a high fluorine (NaF) intake in mature hill bulls kept on a diet adequate in calcium and low in phosphorus, have been studied, and the gross and microscopical lesions in certain of the bones have been described.

2. In connection with pathogenesis, it is pointed out that the ingestion of fluorine causes only a mild disturbance in Ca and P metabolism, due probably to reduced appetite of the animals in an advanced stage of anorexia and consequent inefficient utilization of food.

3. The pathological changes produced, seem to be influenced by the calcioprive and the osteogenic effects of fluorine.

4. Although osteoporosis of the metaphyses of long bones may be said to represent a stage in the development of the final pathological picture, the lesions do not entirely correspond to classical osteomalacia. Certain hyperplastic changes in the diaphysis and metaphysis of the long bones and ribs indicate endosteal activity and the resulting lesions are more suggestive of osteosclerosis than of osteomalacia.

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OCCURRENCE OF RICKETTSIA CONJUNCTIVAE COLES IN GOATS IN THE PUNJAB

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(With Plate XX and two text-figures)

SINCE Coles [1931] described *Rickettsia conjunctivae* as the cause of irido-cyclitis in South African sheep, several workers have recorded its occurrence in many other parts of the world. A closely allied and perhaps identical organism was later described by Coles [1935] as a cause of ophthalmia of goats in South Africa. It is noteworthy that the goat parasite has not been encountered by the several workers who have

observed ocular rickettsiosis of sheep. This disparity may not only be due to the relatively smaller number of goats in many countries but also because the pathogenic effects of this protozoa are not so severe and manifest in goats as they are in sheep.

In India *Rickettsia conjunctivae* was recently encountered by Nanda and Abdussalam [1943] and considered to cause contagious keratitis in

sheep and a closely allied or identical organism was found by Abdussalam [1942] in smears from the inflamed conjunctiva of buffaloes in Lahore. So far as I have been able to ascertain, there is no previous record of the occurrence of a *Rickettsia* in the eyes of goats in India.

While making a clinical examination of a goat at this college in August, 1942, trachoma-like lesions were noticed on the conjunctival lining of the lids and the nictitating membrane. A Giemsa-stained smear from the lesions showed a heavy infestation of the conjunctival cells with a species of *Rickettsia*. Twenty-four other goats of the same type were subsequently examined and found to harbour a similar *Rickettsia*. These goats of the *Malvi* breed, were purchased in the local market but the herd arrived from South Eastern Punjab and nearby Indian States. Their ages varied from 12 to 15 months.

CLINICAL

The twenty-five goats showed trachoma-like lesions in both eyes. These consisted of dull-red nodules varying in size from that of a poppy seed to a boiled sago grain. Several of them were confluent and disposed in a band extending along the margins of the lids. Several of the nodules were also present on the nictitating membrane. When the conjunctival surface was gently rubbed with cotton-wool wetted with saline the nodules turned bright-red and with a little more friction began to bleed. The remaining conjunctival surface was pale roseate in most cases but rarely showed signs of mild catarrh. In these cases there was an accumulation of muco-pus at the inner canthus but in most cases there was nothing but a small quantity of discharge dried along the margins of the eyelid. There was no evidence of keratitis, of impairment of eyesight or of any general illness.

Coles [1935] observed 'acute conjunctivitis and keratitis' in one group of cases and 'ophthalmia' in another. The affection as observed

in the Punjab goats is much milder. Possibly, however, these *Malvi* goats had suffered from a more acute form of the disease in their childhood and this had reached a chronic and milder stage by the time they were brought to Lahore. Unfortunately, transmission experiments could not be carried out, as unaffected goats could not be made available; in any case newly-born kids may have to be employed for this purpose. However, it seems reasonable to suppose that the infection is directly transmissible from goat to goat as is the case with a similar affection in other animals (sheep, buffaloes, cattle, etc.).

THE RICKETTSIAL ORGANISM

Technique of examination. The nodular surface of the affected conjunctiva was gently scraped with the edge of a well-ground slide and the material so obtained smeared on another slide and dried in the air. After fixation for one minute in absolute methyl alcohol and drying, it was stained by Giemsa method (one drop of stock stain per c.c. neutral distilled water, for one hour) or with May-Grünwald-Giemsa. With a little practice, the infected conjunctival cells could be easily seen under low power but the organism itself could only be seen with the 1/12 lens.

Description of the organism. The organism (Fig. 1) is markedly pleomorphic and one can observe round or avoid solid forms, ring forms, horse-shoe or imperfect ring forms, triangles with accumulated masses of chromatoid material at the somewhat rounded angles and bipolar form (mostly with asymmetrical sides). The halos in all but the solid forms may not be empty spaces but only chromophobic parts of the protoplasm as observed by Jackson [1931] in the case of *Rickettsia ruminantium* Cowdry. There is also a wide variation in the dimensions of the individual elements; at their widest points they were found to measure from 0.5μ to 1.4μ . In the material at the writer's disposal the forms with 'halos' (rings, horse-shoes, triangles and bipolars) were relatively more frequent than the solid ones.



FIG. 1 A schematic drawing of the various forms of *Rickettsia conjunctivae* of caprine origin.

In the conjunctival smears examined 40 to 60 per cent of the epithelial cells were found to be infected. Many of the cells were tightly packed with the organism, while others contained a few elements scattered all over the protoplasm (Plate XX, figs. 1 and 2) in most of

the cells clumps of *Rickettsia* were seen but they did not show a tendency to hug the nucleus as is the case in conjunctival rickettsiosis of pigs [Unlenhuth and Haendel, 1913] and of man [Heymann, 1913]. Occasionally one finds small clumps of the organism free in

the smear but this location seems to be due to breaking down of infected cells during the preparation of the smear.

The organism stains pale-blue or lilac blue with Giemsa. The chromatoid masses and the 'poles' however, show a reddish hue. With Pinkerton's method as described by Coles [1940] the protista stains light blue. It is gram negative and non-acid-fast. As with conjunctival rickettsiosis of other animals, it was noticed that the heaviness of the infection was directly proportional to the number of neutrophils in the smear; abundance of these cells always indicated, and was associated with, a heavy infestation of a large number of conjunctival cells.

It is evident from the above description that the organism encountered in the Punjab goats is identical in its morphological, tinctorial and other characters with the *Rickettsia* described by Coles [1935] from goats in South Africa. In the present work the causal relationship of the *Rickettsia* observed to the lesions has been accepted as a working hypothesis. In this connection the following recent opinion of Beveridge [1942] may interest the reader. He states that 'although it has not been possible to prove that *R. conjunctivae* is the cause of contagious ophthalmia the circumstantial evidence is so strong that it seems justifiable to accept its causal role as a working hypothesis.'

ANOTHER RICKETTSIA-LIKE ORGANISM

Coles [1931, a] described what appeared to be another rickettsia-like protista under the heading 'An unknown Intra-cellular organism of the Conjunctival Epithelium of Sheep' from South Africa. The same organism was recorded by Donatien and Lestoquard [1937] in Algeria, but Nanda and Abdussalam (*Loc. cit.*) did not encounter it in Indian sheep. In goats, Coles [1935] recorded the finding of an identical or a closely allied organism in association with the species of *Rickettsia* described above. I have also encountered this protista in two of the twenty-five goats examined and the findings are briefly reported below:

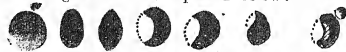


FIG. 2. A schematic drawing of the various forms of the second *Rickettsia*-like organism in the caprine conjunctiva.

This organism is not so highly pleomorphic as *Rickettsia conjunctivae*, the majority of elements being round or ovoid. The other shapes

encountered are shown in Fig. 2. In size, it is larger than *R. conjunctivae* and measures 0.5μ to 1.4μ by 0.4μ to 1.5μ .

The organism is essentially intracellular but it is not uncommon to see some free-borne elements near the infected cell which are probably derived from it. There is no tendency towards clumping and the individual elements lie separately and evenly distributed in the cell protoplasm. The number of individuals in an infected cell is relatively much smaller than with *R. conjunctivae*. (Plate XX, fig. 3).

With Giemsa, the organism takes a blue stain which is somewhat deeper than that of *R. conjunctivae* but is decidedly fainter than that taken by bacteria; there is no reddish tint. As a rule the whole of the protoplasm is not uniformly stained there being a deeper-stained part arranged more or less in the form of a crescent or a half moon.

It has not been possible to make any observations on the pathogenicity of this protista, as on the two occasions on which it was seen, it was associated with *R. conjunctivae*, and the clinical picture in these goats did not differ from that in the others infected with *R. conjunctivae* alone. Coles [1935] however, considered it to be the cause of a mild, conjunctival catarrh.

SUMMARY

1. A conjunctivitis of goats, associated with *Rickettsia*, has been recorded apparently for the first time in India.

2. The lesions observed closely resembled those of trachoma and were, perhaps, a sequel of some more acute stages of the affection suffered by the goats during their kidhood.

3. The method of examination and morphology of the causal organism has been described; it seems to be morphologically identical with *Rickettsia conjunctivae* Coles.

4. Another rickettsia-like organism morphologically identical with one described by Coles [1931, a] was also found in two of the goats. Its presence did not seem to add to the pathogenicity of *R. conjunctivae* with which it was associated.

ACKNOWLEDGEMENTS

The writer wishes to express his gratitude to J. S. Garewal, Esq., I.V.S., Principal of this college, for encouragement and facilities, and his appreciation of the assistance rendered by M. Ata Ullah Khan in the Laboratory and by Mr Kewal Krishan in the preparation of photo-micrographs.

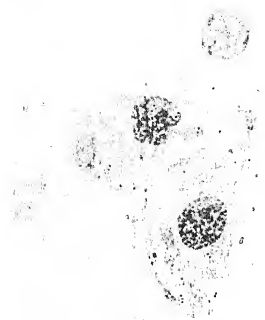


FIG. 1. *Rickettsia conjunctivae* in the conjunctival epithelial cells of a goat; two infected and one clean cell is shown in the field.



FIG. 2. *Rickettsia conjunctivae* in the conjunctival epithelial cells of a goat. Notice the presence of neutrophils in the field.

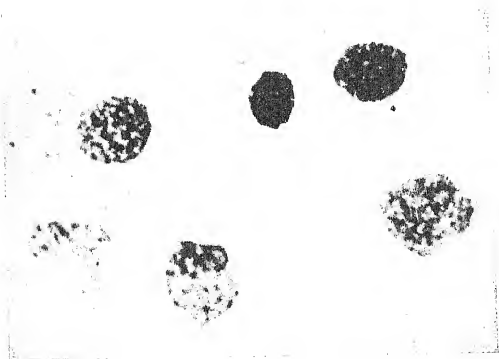


FIG. 3. Rickettsia like organism (unknown intracellular organism of calves) in the conjunctival epithelial cells of a goat; one cell in the top left hand corner is infected.

Note :—Fig. 3 is more highly enlarged than the others

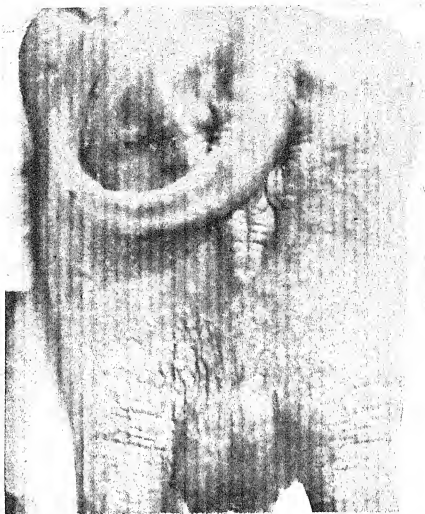


FIG. 1. Buffalo No. 1

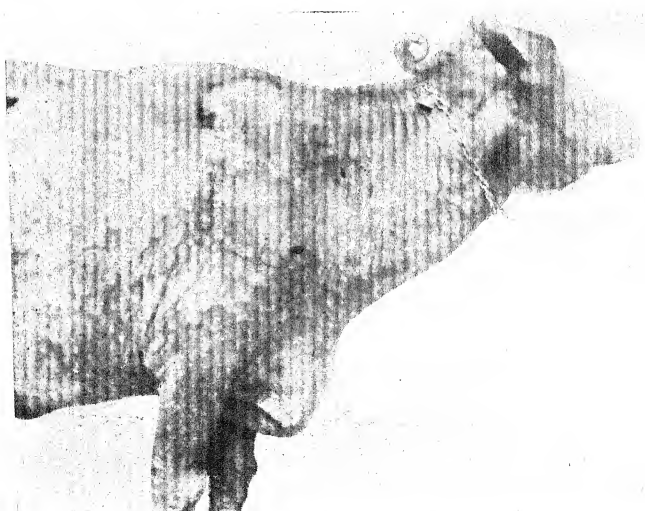


FIG. 2. Buffalo No. 1

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GENERALIZED VACCINIA IN BUFFALOES

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(Received for publication on 1 November 1943)

(With Plate XXI)

VACCINIA is a common, comparatively benign contagious disease chiefly of milch cattle, the lesions of which are mainly localized on the teats and udder. The disease more rarely appears in a generalized form, when eruptions also develop on other parts of the body, e.g. medial aspect of the thighs, quarters, abdomen, chest, neck, muzzle and around the nostrils. From observations recorded by Sharma [1934], it is evident that generalization of pox lesions is comparatively more common in buffaloes than in cows.

Two cases of generalized vaccinia in cows have been recorded by Dupuis [1899], and one case by Strebel [1892]. In these cases the eruptions were present on the teats, udder, inner side of the thighs, croup, rump, lower abdomen, chest, neck and muzzle. According to Wallis Hoare [1913] rarely the pox lesions may spread to other parts of the body such as head, inner surface of thighs, neck, chest, etc. Bidault [1927] says that the lesions of cow pox are almost invariably localized on the teats and udder and do not involve the mucous membranes. Blaxall [1930] also states that generalization of the eruptions is very rare and when present they appear on the head, at the base of the horns and on various parts of the integument. Gaiger [1932] states that the lesions of vaccinia in cows are most frequently found on the teats and udder, in calves on the lips, muzzle and around the nostrils and in bulls often on the scrotum and inside the thighs. Sharma [1934] in India recorded an outbreak of generalized vaccinia, involving 250 cattle (215 buffaloes and 35 cows), in which the eruptions were mainly present on the teats, udder, medial aspect of the thighs and vulva, and in some

cases on the quarters, lips and around the nostrils. Idmani [1943] states that in cattle the lesions are mainly localised on the teats and udder.

Studies of the available literature show that there is no record, in cattle, of pox lesions occurring over the entire body. In the present cases the eruptions developed practically all over the body, being visible on the udder and teats, medial aspect of the thighs, quarters, croup, rump, tail, abdomen, chest, fore-leg, neck, outer surface of the ears, head, around the nostrils etc.

CASE REPORTS

1. A black Murrah buffalo-cow age about seven years, calved on 27 February, 1943.

The animal was examined on 3 May, 1943 in the College Hospital to which it was admitted. The history of the case, as narrated by the owner, was that two days previously small pimples were noticed on the hinder parts of the animal, especially on the teats and udder, where they were present in large number. As a result she kicked badly during milking. Her milk secretion had decreased and she was not feeding or ruminating. There was no history of previous disease. It was stated that another buffalo at a neighbouring house was suffering from a similar disease. The small pox hospital of the Lahore Corporation is situated about half a mile from these houses. On examination the buffalo was found in good bodily condition but was dull and depressed. The temperature was 104.0°F. and the conjunctivae were congested, with watery discharge from both eyes. The muzzle was dry and examination of the mouth revealed nothing abnormal. The udder was hot

and sensitive and the teats slightly swollen. A large number of papules about the size of a lentil were seen on the teats and udder. Milk from all the four quarters was normal in appearance. Papules were also present on the medial aspect of the thighs, tail, quarters, croup, abdomen, and fore-legs (Plate XXI, figs. 1 and 2). The following day she was greatly depressed, temperature was 101.2°F . and she took no food. Papules had also developed on the chest, neck, head, external surface of the ears and around the nostrils. By the 5th day the papules had developed into vesicles which ultimately became depressed in the centre. The temperature receded to 100.8°F . and the buffalo began to feed. The vesicles gradually increased in size till about the 9th day, attaining the dimensions of a hazel-nut, assuming an elliptical shape on the teats and being circular on the udder and other parts of the body. On about the 10th day the vesicles were converted into pustules, which on some parts of the body such as the abdomen and the legs became confluent, forming irregular patches. Gradually all the pustules dried up, forming yellowish scabs which finally changed into thick dark-brown crusts. It was noticed that the vesicles on the teats did not pass through the typical stages of pox, as they were ruptured by the milker during milking, so that the scab stage developed quickly. By the 19th day most of the crusts on the body had become detached, leaving the underlying areas of the skin pinkish in colour and slightly depressed in the centre. The animal was now feeding well and was discharged. The buffalo was again examined on 22 June, 1943. She was then in good condition and the milk yield had returned to normal. Practically all the depressed pinkish areas on the skin had disappeared, and the integument was now normal in appearance.

2. A black buffalo-cow, age about eight years, calved on 2 April, 1943. The history of the case was that on 28 April, 1943, numerous small pimples had been noticed on the teats and udder and that within four days they appeared on the entire body. She took no food and milk secretion had practically ceased. The buffalo was examined on 6 May, 1943. The pox lesions in this case too developed practically all over the body. The animal recovered after about four weeks, but the vesicles on the teats, which were ruptured during milking, ultimately became indolent ulcers. No permanent pock-marks were seen on the skin.

3. A black Ravi buffalo-cow, age about five years, calved about four months previously, was

examined on 6 May, 1943. Pox lesions were present on the teats, udder, medial aspect of the thighs, quarters, vulva, tail, abdomen, chest and around the nostrils. The animal recovered after about three weeks. The depressed areas on the skin gradually disappeared.

4. An aged black Ravi buffalo-cow, calved on 23 March, 1944, was brought to the College Hospital on 19 May, 1944, with the history that five days previously numerous small pimples were noticed on the udder and teats and inside the thighs. They continued to appear on other parts of the body until finally the whole surface was covered. The animal was dull and she neither fed nor ruminated. Milk secretion had much decreased. Two weeks previously the buffalo suffered from rinderpest and had recovered in hospital. The owner mentioned that another buffalo was similarly affected on the adjoining premises, which are situated about three furlongs from the Punjab Vaccine Institute, where small-pox vaccine is manufactured on a large scale. On examination, the buffalo was emaciated, dull and depressed. The temperature was 103.8°F ., and conjunctivae congested and coated with a mucoid discharge. Papules, the size of a split pea, practically covered the body surface, while on the udder and teats a number of vesicles were also present. The papular, vesicular, pustular and scab stages lasted for about five, four, four and seven days respectively. All the dried crusts except a few on the abdomen, became detached, leaving the udder-lying areas of skin pinkish in colour and slightly depressed at the centre. The animal then looked bright and was feeding and ruminating well, but emaciated. The animal was again examined on 2 August, 1944. She was then in good condition and the depressed areas on the skin, except a few on the chest and abdomen, had practically disappeared.

5. An aged black buffalo-cow, calved on 20 March, 1944. The history as narrated by the owner, was that on 10 May, 1944 small pimples about the size of a split pea were noticed on the teats, udder and inside the thighs and that within three days they became generalized over the body. She was not feeding or ruminating while milk secretion had practically ceased. The buffalo was examined on 19 May, 1944. Vesicles with depressed centres were present over the body surface, while on the teats there were a few soft yellowish scabs and on the udder a number of pustules. The vesicles developed into pustules which dried ultimately to dark brown crusts. The animal recovered in about

26 days. Most of the depressed areas on the skin gradually disappeared.

COURSE OF THE DISEASE

In uncomplicated cases it seems that animals usually recover within three to four weeks. In some cases there have been observed secondary complications such as mastitis, stenosis of the milk ducts and formation of indolent ulcers on the teats etc. and these of course prolong the course of the disease.

DIAGNOSIS

The occurrence of eruptions on the teats, udder and other parts of the integument, the fact that they passed through the various classical stages of pox, viz. papule, vesicle, pustule and scab, as well as the evidence mentioned in the next section concerning the origin of the infection, may be regarded as giving a clear diagnosis. That being so, no experimental inoculations on a calf or rabbit were judged to be necessary. A detailed work especially on the characters of the virus is being carried out at this institute.

EPIZOOTIOLOGY

In India it is probably exceptional for employees who are engaged part-time for milking privately-owned animals to wash their hands before milking each animal. Hence transmission of any disease, such as pox, is easy. In an ordinary outbreak of cow-pox the most common source of infection is the importation of new cows, either carrying lesions or in the incubation stage. Blaxall [1930] states that a number of cow-pox outbreaks have occurred after recent vaccination of a milker or attendant who obviously acted as the source of the disease. On the other hand, a common experience has been that the source of outbreaks is not discoverable. Ceely [1940] who investigated numerous outbreaks of cow-pox could not determine the origin of infection and concluded that the outbreaks were spontaneous.

The following facts were ascertained regarding the cases under discussion. Cases Nos. 1, 2 and 3 were tethered side by side during the day under a large shady tree and there was no other cattle in the vicinity. The owner of case No. 2 stated that his children and the servant who looked after the buffalo had been vaccinated against small-pox on 24 April, 1943, i.e. four days before lesions were noticed on the animal. The owner of the case No. 1 had employed a part-time milker, who had also milked buffalo No. 2 which at the time was suffering from

generalised cow-pox. The infection in No. 1 is thus readily explained. Case No. 3 evidently contracted vaccinia by direct contact from the diseased buffaloes. At the time of occurrence of cases Nos. 4 and 5 there was an outbreak of small-pox at Lahore and in consequence numbers of human beings were being vaccinated. The milker who used to milk these two buffaloes had a son who died of small-pox on 15th April, 1944. The milker himself developed a few pustules rather smaller than a split pea on the dorsal surface of the hands between the thumb and the fore-finger and about the wrists. These dried up forming scabs which were removed by him after about 10 days. The source of infection in these two buffaloes is thus also clear. Finally it may be mentioned that other buffaloes were examined in the same locality but none of them was found to be suffering from vaccinia.

SUMMARY

1. Five cases of generalised vaccinia in milch buffaloes are recorded, with eruptions developing practically all over the body surface.
2. The general symptoms were more severe and prolonged than those of classical cow-pox, in which the symptoms are benign and the lesions localised.
3. All the cases occurred at a time when numbers of people were being vaccinated against small-pox and at a time when this disease was prevalent. The source of infection in the animals could thus be readily explained.

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TRANSMISSION OF RINDERPEST BY EXPIRED AIR

By J. A. IDNANI, Imperial Veterinary Research Institute, Mukteswar

(Received for publication on 8 March 1944)

(With one text-figure)

SEVERAL hypotheses have been put forward from time to time to explain the mode of transmission of rinderpest under natural conditions, but none accounts satisfactorily for all or most of the observed experimental data.

Artificially, the disease is readily inoculable with infected materials by all recognized routes, but it is still doubtful what particular route or whether some combination of routes is responsible for the entry of virus into the system under field conditions in natural outbreaks. The highly infectious nature of the disease suggests that rinderpest spreads by contact, but experimental evidence perhaps points to the fact that under natural conditions dissemination may take place through more than one route. Cooper [1932] remarks that "rinderpest spread by direct contact from artificially infected to healthy susceptible cattle at a surprisingly low rate".

The role of arthropods as possible transmitting agents in disseminating this disease has been extensively investigated. Curasson [1922] experimented with *Ixodes ricinus* and with a species of *Tabanus*. He proved the presence of virus in a tick engorged on an infected animal on

the second day of fever by inoculating the macerated tick suspension into a susceptible animal, but when the tick was ground up an hour after removal the virus was inert. With Tabanids the results were negative. Sen [1925] carried out transmission experiments through the agency of *Aedes (Stegomyia) albopicta*, *Musca domestica* and *Linognathus vituli* but the results were entirely negative. Bhatia [1935] employed *Tabanus orientes* and *Stomoxys calcitrans* and in one instance he obtained positive results with the former. With the latter species, however, the results were negative.

Hornby [1926] made a series of controlled experiments to study the paths of infection. He showed that (a) intact skin is impermeable to virus, while broken skin readily admits it; (b) infection can be set up with virulent blood inoculated intradermally, subcutaneously intravenously, or intraperitoneally; (c) the disease is experimentally transmitted through the agency of *Glossina morsitans*; (d) infection can take place occasionally by ingestion when large quantities of infected blood are fed, and (e) infection can be produced by the respiratory tract, viz., by drenching through the nostrils, by intratracheal injection or by swabbing the nasal mucous

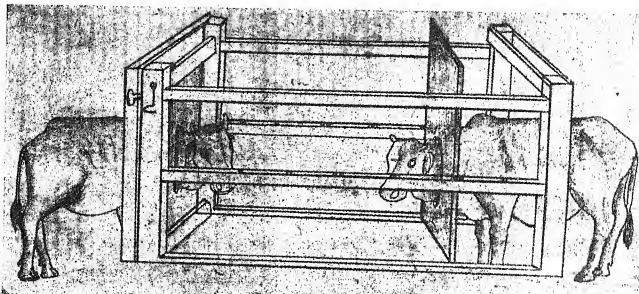


FIG. 1. Transmission of rinderpest by expired air

membrane with infected materials. Hutyra and Marek [1938] suggest that "the infectivity of the expired air is at least questionable".

In this paper are described a series of experiments to test the infectivity of the expired air of bulls inoculated with rinderpest.

METHODS AND MATERIALS

To produce optimum conditions under which expired air from an infected animal would be inhaled by a healthy one a wooden trevis was improvised to secure the experimental animals (Fig. 1). At each end a sliding trap door was provided to secure the animals by the neck, so that they could be made to stand facing each other. The neck trap at one end was movable so that the distance between the animals could be adjusted as required. The whole structure was covered with tarpaulin to allow reasonable ventilation.

Prior to experimentation, all animals used were trained to stand in the trevis without fidgeting for six hours with an interval of two hours for food and rest.

The viruses used were (a) a highly virulent field strain, termed line E, (b) a strain, termed line D of lower virulence and (c) a low virulent goat-adapted strain. Line E was obtained from an outbreak in a Village near Mukteswar in 1939, and it has since been maintained by monthly passage through the medium of spleen tissue collected on the fifth day after inoculation. In the interval the virus is stored in the refrigerator. The mortality among hill bulls treated with this strain is over 90 per cent. Line D has been maintained at the Institute for about twenty years and now causes approximately 12 per cent deaths. The goat-adapted strain was evolved at this Institute in 1927 by implantation of rinderpest virus of bovine origin on the foetal tissues of pregnant goats and then recovering from maternal blood.

After exposure in the experiments, each bull was kept in a chuppur for individual isolation.

The influence of relative humidity as a factor in the transmission of rinderpest by breath was investigated. It was found that the difference between the relative humidity of atmosphere outside and inside the trevis was 10 per cent when a normal bull was exposed; but when a bull infected with the virulent strain was allowed to breathe for six hours the difference was 6

per cent. With a bull infected with the less virulent strain the difference was 8 per cent.

TRANSMISSION EXPERIMENTS

Hall [1935] in a series of tests carried out to compare the virulence of blood with that of nasal secretion taken at the height of temperature found that whereas the minimum quantity of blood capable of causing infection at that time was 0.002 c.c., nasal secretion in 1/10th of that amount was infective. At this Institute blood for experimental purposes is taken on the fourth day after inoculation, if the temperature reaction is typical. The first transmission experiment was therefore commenced on the fourth day after inoculation. On this day a healthy bull was exposed for three hours to an infected animal at a distance of ten feet. No reaction ensued during the next 13 days. The same animal was then re-exposed to another infected bull on two consecutive days (4 and 5) for four and six hours respectively, the distance between the two being reduced to five feet. Five days after the second exposure a rise in its temperature was recorded and the animal subsequently showed symptoms of rinderpest. These experiments showed that rinderpest can be transmitted by the breath and that in the first trial the exposure was too short or the distance too long (Table I).

In the next attempt a healthy bull was exposed to an infected animal on the fourth and fifth days for four and six hours respectively at a distance of ten feet. No infection occurred and the healthy bull later proved susceptible to test with virulent blood. This result indicates that the distance between sick and susceptible animals has something to do with the dissemination of cattle plague. At a further trial at a distance of eight feet between a healthy and an infected animal on the fourth day after inoculation, the disease was not transmitted after an exposure of six hours.

After these preliminary trials, an investigation was designed to limit the three factors, viz. stage of fever, period of exposure at which rinderpest can be transmitted through expired air, and distance. As to the stage of fever it is known from many tests with virus-producers that the first rise of temperature occurs on the third day. Healthy bulls were therefore exposed—from the third to the sixth day, exposure and distance being kept constant, viz. at six hours and six feet (Table I).

Transmission of Rinderpest by Expired Air

TABLE I

Transmission experiments by the breath

Expt.	Date	No. of healthy bulls exposed	Day on which infected bull was exposed	Distance (feet)	Period of exposure (hours)	Result
<i>High virulent virus</i>						
1941						
1	August 8	1	4	10	3	Neg.
2	August 22, 23 . . .	2	4 and 5	10	4 and 6, resp.	Neg.
3	August 29, 30 . . .	1	4 and 5	5	4 and 6, resp.	Pos.
4	November 12	3	4	6	6	Neg.
5	November 27	4	3	6	6	Neg.
6	November 28	5	4	6	6	Neg.
7	November 29	6	5	6	6	Pos.
8	November 30	7	6	6	6	Pos.
1942						
9	June 6	8	5	6	6	Pos.
10	June 7	9	6	6	6	Pos.
11	June 24	10	5	6	3	Neg.
12	June 25	11	6	6	3	Neg.
13	July 4	12	5	6	3	Neg.
14	July 5	13	6	6	3	Pos. (late reaction)
1943						
15	March 30	14	3	6	6	Neg.
16	April 1	15	5	3	6	Neg.
17	April 2	16	6	3	6	Neg.
18	May 7	17	7	6	6	Pos.
19	May 25	18	8	6	6	Pos.
20	June 18	19	9	6	6	Pos.
21	July 4	20	10	6	6	Pos.
<i>Low virulent virus</i>						
1942						
1	October 30	1	5	6	6	Neg.
2	November 17	1	6	6	6	Neg.
1943						
3	March 6	2	5	6	6	Neg.
4	March 7	3	6	6	6	Neg.
5	August 28	4	6th to 9th	6	6	Neg.
<i>Goat-adapted virus</i>						
1943						
1	July 13	1	6th to 9th	6	6	Neg.
2	September 15	2	6th to 9th	6	6	Neg.
3	October 21	3	6th to 9th	4	6	Neg.

It would appear from Table I that an exposure of six hours at a distance of six feet is effective for the transmission of rinderpest by the breath on the fifth and sixth days after artificial infection (Expts. 4-10).

The next point was the minimum exposure at which transmission would be effective. Hence, the period was reduced to three hours, keeping the distance between the sick and susceptible animals at six feet. Expts. 11 to 14 show that in one case out of four infection took place from the sixth day animal after an exposure of three hours. Expts. 15 to 17 suggest that infection is unlikely to take place (a) after six

hours exposure on the third day after infection at a distance of six feet or (b) on the fifth or sixth days at eight feet with a maximum exposure of six hours.

Concluding experiments were then designed to furnish information as to the stage at which an animal with rinderpest ceases to be infective through its expired air. It would appear from expts. 16 to 21 that under the conditions described in this paper the expired air from a rinderpest animal can be infective from 5 to at least 10 days after artificial inoculation. The end-point of the experiment could not be

reached because no animals infected with the virulent strain of rinderpest virus lived beyond 10 days.

An experiment was made to test the role of breath as transmitting agent in a closed atmosphere without direct transpiration. A hill bull artificially infected with line B rinderpest virus was allowed to breathe in the closed trevis within a length of six feet on the sixth day after inoculation. The infected animal was removed after six hours and a healthy bull was secured in its place and detained for 15 minutes. This animal developed typical rinderpest and died on the 12th day.

Less virulent strain

The strain used was the line D virus, mentioned above. The first experiment was commenced on the fifth day at a distance of six feet, the exposure lasting six hours. The healthy animal so exposed remained unaffected for the next 26 days and was then re-exposed to an infected animal on the sixth day under similar conditions. No reaction ensued during the following 13 days and the animal reacted on test. Evidence confirming these findings was obtained in three other experiments with line D virus. In the first two of these, two healthy bulls were exposed, one on the fifth and one on the sixth days, while a third was exposed daily from the sixth to the ninth day. The exposure in all cases was for six hours at six feet, but no transmission occurred and all experimental animals reacted on test.

Goat-adapted strain

In view of the large-scale vaccinations against rinderpest being carried out in India with 'goat vaccine' virus, the infectivity of the expired air of a bull inoculated with goat virus was tried. A healthy animal was exposed to an infected bull daily from the sixth to the ninth day for a period of six hours at a distance of six feet. The disease was not transmitted and the exposed bull later proved susceptible. Similar results were obtained in two more such experiments, although in one case the distance was reduced to four feet.

DISCUSSION

Certain viral and bacterial diseases of man and animals, such as influenza, tuberculosis, contagious pleuropneumonia of bovines and distemper of dogs, are known to be communicable through the nasal chambers and preventive measures in such diseases primarily consist of segregation of victims. Of the many problems that confront the veterinary worker in the field there is none so important as that of the spread

of disease and a thorough knowledge is necessary of the means by which dissemination takes place. In the case of rinderpest, experimental evidence brought out in this paper, shows that expired air of infected animals can play a part in spread and may explain the contact infections recorded in the literature. This finding further strikes a note of caution to those engaged on researches of a similar nature to avoid vitiation of their experiments by this hitherto unknown route.

Such conditions, it must be admitted, as have made it possible to prove the role of breath as a transmitting agent in rinderpest, are perhaps unobtainable in all outbreaks of the disease, but it cannot be denied that in most of the rural areas in India cattle are housed in narrow enclosures and through lack of proper understanding of animals hygiene, sick animals are frequently kept together with the rest of farm stock, thus facilitating dissemination of disease.

The differing behaviour of the virulent and the two less virulent strains of virus raises the much discussed question of dose and virulence as factors in the successful implantation of a virus on susceptible tissue.

The existence of three factors, viz., distance, period of exposure and stage of the infected animal, which determine whether transmission is or is not successful, at any rate in the case of line E virus, supports the view that much diffusion of the infective agent takes place in the air currents between an infected and a healthy animal. The problem of the degree of subinfective dosage required to initiate an infection is worthy of assessment, but for financial reasons even a preliminary test to compare the virulence of line D virus with that of line E virus in terms of minimum infective dose has been withheld for the time. The present small investigation was limited to the question of whether rinderpest can be shown to be easily communicable by the expired air of infected animals. Now that this has been established, the examination of details can be undertaken as facilities permit.

Much work has been done to explain airborne infections of man. Thus Dudley [1928], in defining the infective range of droplets expelled from the nose or mouth, states that some of these droplets are visible and contain many cells and hundreds of bacteria, while others are of microscopical dimensions with only a single cell and one or two bacteria. Heavy particles drop to the ground within two or three feet as the propulsive force of expiratory effort ceases, but smaller droplets may remain suspended for some time according to the atmospheric humidity

and temperature and may be carried to almost any distance according to the force and direction of the prevailing air currents. This explains why contact must be fairly close in order to catch an infectious disease. A fine droplet mist, though more persistent, is generally too dilute to do harm. Thus dosage of infective material is an important factor in transmission of air-borne diseases. The effective dose also varies relatively and actually with every member of the herd and with every species and strain of bacteria, because each individual has a special resistance which can deal with particles of the infective agent below a certain minimum but variable number.

Most field strains of rinderpest are presumably of a highly virulent nature and are associated with high mortality and their wide prevalence is probably at least partly dependent on such factors as are discussed in this paper. On the other hand, it may be noted that so far there is only one outbreak of rinderpest on record—and that of very mild intensity—which could be attributed to goat virus, a remarkable fact in view of the wide spread use of goat-adapted virus for immunizing cattle in India. This tends to support the observations above on the transmission through the breath of the less virulent goat-adapted virus in bulls.

SUMMARY

Hill bulls infected with a virulent strain of rinderpest virus are capable of transmitting the disease by the expired air when exposed to healthy animals. Under the experimental conditions used a minimum of six hours exposure

and a distance of six feet between infected and healthy animals were necessary for positive results. The breath of bulls artificially infected with the virulent strain is infective by the fifth day. When, however, air in a semi-enclosed space become charged with virus, a sufficient quantity to infect may be taken up in at most 15 minutes by a susceptible animal breathing that air.

Rinderpest was not transmitted to bulls under normal conditions either by a less virulent strain or by goat-adapted virus.

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STUDIES ON THE DETERMINATION OF DIGESTIBILITY COEFFICIENTS

III. SINGLE TRIAL METHOD FOR THE DIRECT ESTIMATION OF INDIVIDUAL DIGESTIBILITY COEFFICIENTS IN A MIXED RATION*

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The estimation of the respective shares of digestibility of individual feed and feed components in a mixed ration offers a problem which so far has been only partially solved. The matter is much simpler when it involves a single feed, since, by assuming the faeces as indigestible residue, the amount apparently digested can be calculated by the difference. When, however, two or more feeds are concerned, the possibility of interaction between one feed and another involves a deviation, the extent of which is still unknown.

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In such cases the usual procedure of estimation is by what may be called "the method of elimination". It dates back since its inception about 1860 by Hennberg and Stohmann and is still in almost universal use. It requires one, two or three successive feeding and metabolic tests, depending on the number of feeds used. The first trial is conducted with one feed, and, when the digestibility of this feed is thus ascertained, the second trial is conducted in which this feed is given in combination with the

second, and then by assuming that the digestibility of the first feed remains constant, the value of the second is computed by difference, i.e., by the 'method of elimination'. By a similar process the values of the third or fourth feed are obtained.

In the experiments conducted by Carbery, Chatterjee and Hye [1934], it was pointed out from actual tests that, when an incomplete fodder like rice straw forms the sole feed, the deficiency of nutrients in it is sometimes so seriously reflected in the animal that the values obtained cannot be valid for use in the calculation, by difference, of the digestibility of associated feed or feeds. These difficulties prompted those authors to design a comprehensive experiment involving 18 individual tests in a randomized cycle order and ultimately working at the values directly by graphical method and by regression equation.

While the theoretical background of the method was beyond question, its main difficulty lay in the large numbers of individual tests involving a considerable outlay in men, material, laboratory space and expense. Moreover, the quantity of feed at disposal may not always be sufficient for such an elaborate test. This led to the approach to the problem by a shorter method [Carbery and Chatterjee 1936] in which a simpler formula was worked out on the basis of two individual tests, and the data examined to arrive at a certain minimum standard of statistical accuracy. It was found that, with feeds like rice straw and linseed cake, a fair order of accuracy was ensured with a replication of three to five in the case of the majority of feed components.

It is not, however, possible by either of the two methods to obtain the picture which an individual animal may display, or which may sometimes form the special feature in respect to some particular animal or animals.

PRESENT METHOD

The method described here has originated from these considerations. It aims at adjusting the experimental procedure in such a way that it may be possible to work out the values from the data of a single animal. The fundamental principal involved in calculation is the same, viz. the application of multiple regression equation in which, say,

R represents roughage,

C represents concentrate,

D represents amount digested,

while a and b are digestibility co-efficients of R and C respectively.

Since R, C and D are numerical values, the values of a and b can be worked out by simple simultaneous equation [Carbery and Chatterjee 1936] or by multiple regression equation [Carbery, Chatterjee and Hye, 1934] as is given below:

$$a \Sigma R^2 + b \Sigma CR = \Sigma DR$$

$$a \Sigma CR + b \Sigma C^2 = \Sigma DC$$

Underlying principle

In the previous trials, the regression equation was worked out, as already stated, with the data of a number of animals. Each such datum represented the mean of 10 days reduced to the values of a single day. The modification now suggested is based on the principle that, if instead of taking the mean of ten or more days, each day's performance of individual animals is separately assembled, it will provide from the source of a single animal as many single data as the number of days used for their collection. In other words if the number of experimental days be regulated in due regard to statistical requirement, the data obtained from each animal can validly form the basic material for the purpose of regression equation.

It is, however, necessary for facilitating the numerical side of calculation that the individual data from day to day should vary, otherwise when the equation is worked out the values of same or very nearly same magnitudes will eliminate out.

In so far as the roughage is concerned, such a probability is generally less so long as it is fed *ad lib*. In the case of concentrates which, the animal generally likes and eats whatever is given, the best course is to fix the amount (from day to day) on a random variation which can be very conveniently worked out by a series of varying factors applied on live weights. This simplifies the calculation for individual animals, as the same factors worked on different live weights would indicate the amounts to be fed to individual animals.

The procedure used in the experiment under report was drawn up in due regard to these considerations. In order to enable the formation of a clearer idea an instance of the data used in the final calculation is given in the following table.

TABLE I
Experiment III of 1941
Animal D 6

Days	Distribution of cake			Data used in regression equation.		
	Factor used per lb. of live weight	Live weight lb.	Air dry cake fed (gm.)	Straw nitrogen* (gm)	Cake nitrogen* (gm)	Digested nitrogen (gm)
	X	Y	XY	R	C	D
1st	1.232	854	1052	38.049	66.109	63.824
2nd	1.012	854	864	42.556	55.004	54.182
3rd	1.078	854	920	36.181	58.421	55.294
4th	1.265	854	1080	40.488	58.256	55.142
5th	1.045	854	892	33.748	61.646	54.788
6th	1.100	854	939	40.304	59.904	60.150
7th	0.990	854	845	38.961	53.484	49.434
8th	1.210	854	1032	39.386	60.886	62.796
9th	0.880	854	751	39.677	48.674	46.360
10th	1.276	854	1089	38.266	71.604	63.115
11th	0.968	854	826	35.672	53.693	47.841
12th	1.056	854	901	41.768	37.112	51.360
13th	1.188	854	1014	39.855	65.889	66.885
14th	1.034	854	883	42.303	56.984	51.947
15th	1.166	854	995	36.825	64.574	58.065

* These values have been obtained from chemical analyses, and, although theoretically they should bear a direct relation with the corresponding amount of cake, some variation is implicit, as dry weights and air dry weights of cake from day to day did not vary on a constant proportion. A slight variation is also inherent between the analytical composition of different days' samples.

The amount of air dry cake fed to the animals from day to day was worked out by multiplying the factor X (each day's respective factor) with the live weight X. The values thus obtained have been set up in column four under XY. The corresponding values of cake nitrogen as ingested from day to day have been obtained from the individual analysis of each day, and have been represented in column six under C. These together with straw nitrogen R and digested nitrogen D form the data finally used for regression equation.

The results work out as follows:

$$a = 0.8318 \pm 0.070$$

$$b = 0.8713 \pm 0.1712$$

The co-efficients of correlation between the consecutive day's observation as found by Dr P.V. Sukhatme were as follows:

$$\text{Straw nitrogen} \quad . \quad . \quad 0.40$$

$$\text{Cake nitrogen} \quad . \quad . \quad 0.76$$

$$\text{Digestible nitrogen} \quad . \quad . \quad 0.76$$

The last two are significant at 1 p.e. level of probability and suggest that these method of using individual days' values in place of mean of 10 or more days is not without a defect. However, so far as the digestibility values are concerned the values of a and b, as obtained, here show a reasonable order of agreement with the values obtained by the other method. The statistical order of accuracy has not been as

satisfactory. This appears to be associated with some complexities inseparable with the basic data.

The animals do not work with mathematical precision, and while the variation to this may be a peculiarity inherent with individual animals, the main difficulty lies in respect to the apportionment of the real amount actually falling to the corresponding shares of feeds and faeces. There is at present no satisfactory means by which a correct differentiation of these can be made.

Possibly a solution of it can be found in increasing the number of collecting days and then working out on the basis of a certain specified standard as to what should be the minimum number of days compatible with such a standard of statistical accuracy.

It is also necessary to state here that much remains to be done to bring the method to conform to such a strict standard. But, even if it only indicates a partial approximation to it (as in the present case), it will be a definite improvement over the prevailing "method of elimination" in which altogether assumed values have to be used which do not permit of any form of statistical examination. Besides, no other method contemplates the assessment from the performance of single individual animal from a single course of trial.

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The author is indebted to Dr P. V. Sukhatne, Statistician to Imperial Council of Agricultural Research, Prof. P. C. Mahalanabis, Messrs S. Roy, P. Bose and J. Sen Gupta of Statistical Institute, Calcutta, Mr P. C. Roy, Statistician to Bengal Agricultural Department for help and

STUDIES IN THE BACTERIOLOGICAL AND HANDLED UNDER DIFFER INDIAN CITY (BAN

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(Received for publication on 16

ALL the world over, the hygienic quality of market milk is as a rule assessed by its bacteriological examination, the methods commonly employed being the plate method for total bacterial count, methylene blue reduction test and presumptive coliform test. Extensive work has been done on this subject by foreign workers under their own conditions of supply and this has enabled the milk controlling authorities there to lay down different standards for the bacteriological grading of market milk supplies. This system of classification and control of the milk supply has not only safeguarded the interests of the consumer but has prevented enormous losses to the producer, distributor and manufacturer through the milk going bad.

The quality control of market milk in India today covers only its chemical aspect and aims at checking its adulteration. The bacteriological grading of milk is not in force. The conditions of production, handling and distribution being far from satisfactory, the milk industry at present suffers an enormous loss through souring and other causes and the consumer's health may be greatly imperilled. The first step considered necessary to bring about an improvement in the present state of affairs is the obtaining of information on the bacteriological quality of milk produced and handled under conditions prevailing in this country. Only a very limited amount of work on the subject has so far been done. Joshi [1916] examined 68 samples of milk collected from city stables and 240 samples drawn at random from dairies, cattle stables, milk hawkers and milk shops in Bombay. He found that the former averaged 17,103,000 and the latter 36,385,000 bacteria per ml. Walton [1925, 1927] examined

guide cent.) of individual cows and buffaloes contained under 50,000 bacteria per ml. This also Messured a very large proportion of samples ng under 25,000. The majority of herd Carbles (cow and buffalo) contained between 7,000 and 100,000 bacteria per ml. The pro-Carptions of cow herd samples giving a count Sw and above 100,000 were 91.05 per cent. 8.92 per cent. and of buffalow herd samples 35 per cent and 15.92 per cent respectively. samples of domestic cows gave count upto ,000 bacteria per ml. except for one sixth of total number which gave between 100,000 200,000. No sample of milk from town ,000 stables contained below 100,000 bacteria ml. A little more than half of the total ml. A little more than half of these had between 200,000 and 500,000 and the remaining between 500,000 and millions bacteria per ml. The majority of age milk samples contained between 500,000 sam 10 millions bacteria per ml. and only turent one-sixth of the total number had between the 100,000 and 500,000.

seafhe methylene blue reduction times of 88.66 dur cent. of the samples of individual cows and Insfaloes was about 330 min. (5½ hr.) and of dug over half the number were above 420 min. shohr.). The remaining were decolourized andveen 329 and 240 min. (5½ hr.—4 hr.). 235en separately the reduction time of above V below 330 min. was recorded in case of if 74 per cent and 9.24 per cent. of cow milk deuples and 84.33 per cent and 15.66 per cent thebuffalo milk samples respectively. All the gied milk samples (cow and buffalo) decolour-inal between 240 and 419 min. (4—7½hr.); of oulke more than half of the total number were theween 240 and 329 min. (4—5½hr.), only one theuple being above 420 min. and two below 240 dug. The proportions of the cow herd samples inah reduction times above and below 330 min. thee 44.61 per cent. and 55.38 per cent. and ovebuffalo herd samples 38.93 per cent. and 61.06 inf. cent respectively. All the samples of fromestic cows decolourized between 240 and to) min. (4—7½ hr.); of these a large proportion The between 240 and 329 min. (4—5½ hr.). The uction time of a majority of samples of town ink stables was between 120 and 239 min. (2.4 th), an appreciable number being between 240 ml 329 min. (4—5½ hr.). The number of samples coove 329 min. was negligible. The majority of tilage milk samples decolourized between 60 d 179 min. (1—3 hr.), about two-fifths of the al number were between 180 and 329 min. -5½ hr.) and a negligible proportion below va min. of

TABLE I
Experiment III of 1941
Animal D 6

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* These values have been obtained from chemical analyses, and, although theoretically they should bear a direct relation with the corresponding amount of cake, some variation is implicit, as dry weights and air dry weights of cake from day to day did not vary on a constant proportion. A slight variation is also inherent between the analytical composition of different days' samples.

The amount of air dry cake fed to the animals from day to day was worked out by multiplying the factor X (each day's respective factor) with the live weight X. The values thus obtained have been set up in column four under XY. The corresponding values of cake nitrogen as ingested from day to day have been obtained from the individual analysis of each day, and have been represented in column six under C. These together with straw nitrogen R and digested nitrogen D form the data finally used for regression equation.

The results work out as follows:

$$a = 0.8318 \pm 0.070$$

$$b = 0.8713 \pm 0.171$$

The co-efficients of correlation between the consecutive day's observation as found by Dr P.V. Sukhatme were as follows:

Straw nitrogen . . . 0.40

Cake nitrogen . . . 0.76

Digestible nitrogen . . . 0.76

The last two are significant at 1 p.c. level of probability and suggest that these method of using individual days' values in place of mean of 10 or more days is not without a defect. However, so far as the digestibility values are concerned the values of *a* and *b*, as obtained, here show a reasonable order of agreement with the values obtained by the other method. The statistical order of accuracy has not been as

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It is also necessary to state here that much remains to be done to bring the method to conform to such a strict standard. But, even if it only indicates a partial approximation to it (as in the present case), it will be a definite improvement over the prevailing "method of elimination" in which altogether assumed values have to be used which do not permit of any form of statistical examination. Besides, no other method contemplates the assessment from the performance of single individual animal from a single course of trial.

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STUDIES IN THE BACTERIOLOGICAL QUALITY OF MILK PRODUCED AND HANDLED UNDER DIFFERENT CONDITIONS IN AN INDIAN CITY (BANGALORE)

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ALL the world over, the hygienic quality of market milk is as a rule assessed by its bacteriological examination, the methods commonly employed being the plate method for total bacterial count, methylene blue reduction test and presumptive coliform test. Extensive work has been done on this subject by foreign workers under their own conditions of supply and this has enabled the milk controlling authorities there to lay down different standards for the bacteriological grading of market milk supplies. This system of classification and control of the milk supply has not only safeguarded the interests of the consumer but has prevented enormous losses to the producer, distributor and manufacturer through the milk going bad.

The quality control of market milk in India today covers only its chemical aspect and aims at checking its adulteration. The bacteriological grading of milk is not in force. The conditions of production, handling and distribution being far from satisfactory, the milk industry at present suffers an enormous loss through souring and other causes and the consumer's health may be greatly imperilled. The first step considered necessary to bring about an improvement in the present state of affairs is the obtaining of information on the bacteriological quality of milk produced and handled under conditions prevailing in this country. Only a very limited amount of work on the subject has so far been done. Joshi [1916] examined 68 samples of milk collected from city stables and 240 samples drawn at random from dairies, cattle stables, milk hawkers and milk shops in Bombay. He found that the former averaged 17,103,000 and the latter 26,385,000 bacteria per ml. Walton [1925, 1927] examined

samples of milk produced at the Pusa Agricultural Research Institute Dairy and found that the plate and coliform counts varied with the season of the year, the highest found being during the rainy season. The Imperial Dairy Institute [1939] examined samples of milk produced and handled in its dairy and the results showed that the average plate count per ml. and methylene blue reduction time were 235,000 and four hours respectively.

While the work done so far is interesting, it does not provide the material necessary for defining the quality of milk offered for sale in the Indian market on the basis of its bacteriological condition. The work involved in obtaining material of this kind must of necessity be difficult and considerable, due to various factors, the chief amongst them being the vastness of the country, the conservative methods of production, handling and distribution of milk, inadequate and difficult transport facilities and the variety of climatic conditions prevailing all over the country. It was therefore, felt that information on the quality of milk obtained from different sources was a necessary prelude to further work being done on the subject. The work carried out under this investigation was therefore undertaken. Throughout this investigation the tests employed to determine the bacteriological quality of milk were (a) methylene blue reduction test and (b) the plate count. The work was confined to the conditions governing the supply of milk to Bangalore.

EXPERIMENTAL

The supply of milk to Bangalore is from various sources. The milk sold is either that of cow or buffalo or a mixture of both. The

main agencies supplying milk are:—(i) dairy farms, (ii) travelling milch cows, (iii) town-milk stables a part of which is also retailed through middlemen—distributors; (iv) milk hawkers; (v) town dairies; (vi) the village produced milk, most of which comes in from distances varying from 2 to 20 miles and passes to the customers through dairies, milk being also retailed by hawkers; in the case of a few nearby villages, all or part of the output is retailed by the producer himself; and in addition to the above sources, (vii) some citizens maintain animals for their domestic supply. In order to study the quality of milk obtained from the above sources, samples were taken in each case at the production as well as the supply end. The different sources examined and the conditions of production and handling of milk observed at each source are described below:

(i) *Dairy farm (Institute Farm)*

(a) *Individual animals (cows and buffaloes).* Grooming, washing and feeding were completed about half an hour before milking. Milking was done by hand in a sanitary byre using covered top and sterilized milking pails after the necessary preliminary precautions of washing and disinfecting hands and udders and discarding the foremilk. Samples were taken from the milking pail on the completion of milking and held in a frigidaire cabinet (45°—50°F.) for about $1\frac{1}{2}$ hr. before examination.

(b) *Herd milk (cow and buffalo).* Milk obtained from individual animals was immediately carried to the weighing and collecting room and strained through a cotton filter pad before weighing. After weighing, the milk was transferred to 8 to 10 gallon sterilized cans which were kept closed. Each churn took between 15 and 20 minutes to fill and represented the milk from 5 to 10 animals. Cow and buffalo milks were collected separately. Samples were drawn from the filled cans and held in the frigidaire for about $1\frac{1}{2}$ hr. before examination.

(c) *Dairy milk (cow and buffalo).* The cans of milk, after standing in the milk collecting room for about two hours, were conveyed to the dairy (a distance of about 200 yds.), in a covered hand drawn truck. Samples of cow and buffalo milks were taken separately and immediately examined. Each sample represented the production of about 100 cows and 20 buffaloes.

(ii) *Travelling milch cows.*

In this system of milk supply the owner moves his animal from door to door, draws milk

under the supervision of the customers till the animal is milked dry. The animals appeared to have been washed before being brought in. Udders were washed with water and milking then done in the open on the road-side by hands previously washed with water. Samples for examination were drawn from the milk measures before they were emptied into the customers containers.

(iii) *Town stables*

The stables were of kacheha construction, with poor drainage. The surroundings and the cattle were not cleaned properly. Cleaning of utensils was done by scouring with mud and rinsing out with water. Milking was done in an open top cylindrical milking vessel in an unclean manner. Milk was then collected in containers or cans and samples for examination were taken from these cans each samples representing the production of six to eight animals.

(iv) *Milk hawkers*

The milk hawkers used milk cans made of galvanized iron or aluminium or tinned iron of 2 to 30 lb. capacity. The cans were carried either hanging on bicycle handles or on the head. The cans had no protection against sun and dust other than their loose fitting lids. Samples for examination were taken from the cans between 6 and 8 a.m. when the milk was about two to four hours old since drawn.

(v) *Town dairies*

The dairies from where samples of milk were taken handled between 100 and 500 lb. of milk daily and some of them were situated in busy bazar localities. The equipment for handling milk consisted of galvanized iron or tinned brass drums, buckets and measures. The source of water supply was the municipal tap and no dairy was provided with proper cleaning facilities. These dairies obtained their milk supplies from villages and town milk stables and the milk was therefore, about two to five hours, old when received. Retailing was done at the counter and also at the customers' houses through roundsmen. The method of handling the milk was very unsatisfactory. Samples for examination were taken at the counter between 8 and 10 a.m. when the milk was about four to five hours old.

(vi) *Village milk:*

Samples of mixed milk (cow and buffalo) were taken from villages adjoining the Imperial

Dairy Institute Farm. The houses had tiled roofs, closed sides, kachcha flooring and poor drainage. Well water was used for watering the animals and for cleaning purposes. The cattle houses were merely swept; the manure was allowed to lie nearby. Ordinary vessels were used for milking and handling of the milk and their cleaning done by the indigenous method. The animals were washed occasionally in most cases, their feeding was done during milking and milking was performed in an unclean manner. Samples of bulked milk were taken for examination. Each sample represented the production of about 20 animals.

(vii) *Domestic cows.* The udders of the cows were washed with tap water. After the calves had been sucked, milking was done in brass vessels with small openings. Cleaning of the milking vessels was done by scouring with ash and mud, rinsing out with water and finally draining and drying them in shade. The vessels were rinsed out with the tap water before use. Samples for examination were taken from the milking vessels on completion of each milking.

In this work samples of only morning production and supply were taken. The atmospheric temperatures at the time varied from 75°F. to 85°F. Although the Imperial Dairy Institute sells pasteurized milk, samples of only raw milk before pasteurization were examined to compare the quality obtainable under farm conditions with the other sources of supply. All the samples, other than those taken at the Imperial Dairy Institute cattle yard and dairy, were brought to the laboratory in ice boxes and examined, within about half an hour of sampling, for Methylene Blue Reduction time and bacterial count. The Methylene Blue Reduction test was performed according to Wilson's modified technique [1935] and the bacterial count was determined by the plate method using milk agar as recommended by the Ministry of Agriculture [1934]. A total of 1340 samples taken, both from the production and supply ends was examined. The results obtained are given in Tables I and II respectively.

RESULTS AND DISCUSSION

The results of the tests with the samples at production (Table I) show that, under the conditions obtainable on the Imperial Dairy Institute Farm (representing standard dairy farm conditions), a majority of the milk samples (86.72

per cent.) of individual cows and buffaloes contained under 50,000 bacteria per ml. This also included a very large proportion of samples giving under 25,000. The majority of herd samples (cow and buffalo) contained between 25,000 and 100,000 bacteria per ml. The proportions of cow herd samples giving a count below and above 100,000 were 91.05 per cent. and 8.92 per cent. and of buffalo herd samples 84.05 per cent and 15.92 per cent respectively. All samples of domestic cows gave count upto 100,000 bacteria per ml. except for one sixth of the total number which gave between 100,000 and 200,000. No sample of milk from town milk stables contained below 100,000 bacteria per ml. A little more than half of the total number of these had between 200,000 and 500,000 and the remaining between 500,000 and 5 millions bacteria per ml. The majority of village milk samples contained between 500,000 and 10 millions bacteria per ml. and only about one-sixth of the total number had between 200,000 and 500,000.

The methylene blue reduction times of 88.66 per cent. of the samples of individual cows and buffaloes was about 330 min. ($5\frac{1}{2}$ hr.) and of this over half the number were above 420 min. (7 hr.). The remaining were decolourized between 329 and 240 min. ($5\frac{1}{2}$ hr.—4 hr.). Taken separately the reduction time of above and below 330 min. was recorded in case of 90.74 per cent and 9.24 per cent. of cow milk samples and 84.33 per cent and 15.66 per cent of buffalo milk samples respectively. All the herd milk samples (cow and buffalo) decolourized between 240 and 419 min. ($4-7\frac{1}{2}$ hr.); of these more than half of the total number were between 240 and 329 min. ($4-5\frac{1}{2}$ hr.), only one sample being above 420 min. and two below 240 min. The proportions of the cow herd samples with reduction times above and below 330 min. were 44.61 per cent. and 55.38 per cent. and of buffalo herd samples 88.93 per cent. and 61.06 per cent respectively. All the samples of domestic cows decolourized between 240 and 419 min. ($4-7\frac{1}{2}$ hr.); of these a large proportion were between 240 and 329 min. ($4-5\frac{1}{2}$ hr.). The reduction time of a majority of samples of town milk stables was between 120 and 239 min. (2-4 hr.), an appreciable number being between 240 and 329 min. ($4-5\frac{1}{2}$ hr.). The number of samples above 329 min. was negligible. The majority of village milk samples decolourized between 60 and 179 min. (1-3 hr.), about two-fifths of the total number were between 180 and 329 min. $3-5\frac{1}{2}$ hr.) and a negligible proportion below 60 min.

TABLE I
Results of the examination of the samples taken at production end

Source	Total bacterial counts per ml. of milk	Methylene blue reduction time in minutes							Total No. of Samples	Per cent of each source	
		420 and over	410 to 320	320 to 240	230 to 150	170 to 120	110 to 60	Below 60			
(1) <i>F. D. I. Farm</i> (a) Individual cows	Below 25,000 25,001 to 50,000 50,001 to 100,000 100,001 to 200,000 Total	59 13 9 4	24 39 9 ...	13 3	96 56 18 4	173 ...	55.49 31.79 10.39 2.31
(b) Individual buffaloes	Below 25,000 25,001 to 50,000 50,001 to 100,000 100,001 to 200,000 Total	25 4 3 1	13 16 6 2	7 6	45 29 9 3	83 ...	54.21 31.82 10.84 3.61
(c) Hard milk (cow)	Below 25,000 25,001 to 50,000 50,001 to 100,000 100,001 to 200,000 200,001 to 500,000 Total	...	2 43 5 1	33 53 17	2 102 141 13 1	269 ...	0.74 37.91 52.41 3.55 0.37
(d) Hard milk (buffalo)	25,000 to 50,000 50,001 to 100,000 100,001 to 200,000 200,001 to 500,000 Total	1	13 21 6 9	17 43 9	30 65 15 3	113 ...	26.54 57.51 13.27 2.65
(2) Domestic cows	Below 25,000 25,001 to 50,000 50,001 to 100,000 100,001 to 200,000 Total	...	2 4
(3) Town milk stalls	Below 25,000 25,001 to 50,000 50,001 to 100,000 100,001 to 200,000 200,001 to 500,000 500,001 to 1 million 1 to 5 millions 5 to 10 millions Total	...	9 1 ...	15 27 3	24
(4) Village milk	Below 25,000 25,001 to 50,000 50,001 to 100,000 100,001 to 200,000 200,001 to 500,000 500,001 to 1 million 1 to 5 millions 5 to 10 millions Total	...	1

* Imperial Dairy Institute, Bangalore.

TABLE II
Results of the examination of the samples taken at supply end

Source	Total bacterial counts per ml. of milk range	Methylene blue reduction time in minutes							Total No. of Samples	Per cent of each source
		420 and over	419 to 330	329 to 240	239 to 150	179 to 120	119 to 60	Below 60		
(1) <i>I. D. I. Dairy</i> (a) Cow milk	50,000 to 100,000	...	3	8	11	14.66
	100,001 to 500,000	...	2	28	30	40.0
	500,001 to 500,000	38	1	34	45.33
	Total	...	5 (6.66%)	69 (92.0%)	1 (1.33%)	75	
(b) Buffalo milk	75,000 to 100,000	...	4	10	4	6.66
	100,001 to 200,000	1	20	17	31	51.66
	200,001 to 500,000	...	8	25	41.66
	Total	1 (1.66%)	32 (58.33%)	27 (45.0%)	60	
(2) Travelling milk cows	Below 25,000	...	4	2	6	7.05
	25,001 to 50,000	...	6	21	27	7.05
	50,001 to 100,000	...	6	24	31.75
	100,001 to 250,000	...	2	13	2	24	28.23
	250,001 to 500,000	15	19	22.35
	500,001 to 1 million	3	3	3.55
	Total	...	21 (34.70%)	62 (72.94%)	2 (2.35%)	85	
(3) Milk hawkers	200,000 to 500,000	4	4	5.0
	500,001 to 1 million	10	21	15	...	41	57.5
	1 to 5 millions	1	12	16	1	30	37.50
	Total	15 (18.75%)	33 (41.25%)	31 (38.75%)	1 (1.25%)	80	
(4) Town dairies	1 to 5 millions	8	30	16	49	42.60
	5 to 10 millions	2	12	19	33	28.60
	10 to 20 millions	1	...	13	14	12.7
	Over 20 millions	19	19	16.52
	Total	6 (5.21%)	42 (30.52%)	67 (58.26%)	115	

On the supply side (Table II) 56.29 per cent. of the cow and buffalo milk samples drawn from the Imperial Dairy Institute dairy showed between 50,000 and 200,000 bacteria per ml., including a large proportion between 100,000 and 200,000. The remaining showed between 200,000 and 500,000 bacteria per ml. Taken separately the proportions of cow milk samples below and above 200,000 bacteria per ml. were 54.66 per cent and 45.33 per cent and of buffalo milk samples 58.72 per cent and 41.66 per cent respectively. A majority of samples of travelling milch cows (74.08 per cent) was below 200,000 bacteria per ml. including a large proportion below 100,000. Of the remaining samples, a negligible number was between 500,000 and 1 million bacteria per ml. Very few samples of milk hawkers showed between 200,000 and 500,000 bacteria per ml., the majority falling between 500,000 and 1 million and an appreciable number between one and five millions bacteria per ml. All the samples of town dairies contained above one million bacteria per ml., 71.29 per cent of the samples having between 1 and 10 millions and the re-

maining above 10 millions. The highest recorded was about 92 millions.

The methylene blue reduction times of a majority of samples drawn from the Imperial Dairy Institute dairy were between 240 and 320 min. (4½ hr.). The remaining were above 320 min. except the one sample which decolorized between 180 and 230 min. (3-4 hr.). Almost the same results were obtained with the samples of travelling milch cows. The majority of samples of milk hawkers decolorized between 60 and 179 min. (1-3 hr.) and the reduction times of the remaining were between 180 and 230 min. (3-4 hr.); only one sample decolorized within 60 min. The reduction times of all the samples of town dairies were below 119 min. the majority decolorizing within 60 min. A very small number of samples fell between 120 and 179 min. (2-3 hr.).

The above results as judged on the basis of a majority of samples of each class show considerable differences in the bacteriological quality of milk occurring under the different conditions of production and supply. A general comparison is given in Table III.

TABLE III

Source	Bacterial count per ml.	Methylene blue reduction time
<i>Production</i>		
(1) Imperial Dairy Institute Farm—		
(a) Individual animals	Below 50,000	Over 330 min. (5½ hr.)
(b) Herd	Below 100,000	240 to 330 min. (4 to 5½ hr.)
(2) Domestic cows	Below 100,000	240 to 330 min. (4 to 5½ hr.)
(3) Town milk stables	Between 200,000 and 5 millions	120 to 330 min. (2 to 5½ hr.)
(4) Village	Between 500,000 and 10 millions	60 to 240 min. (1 to 4 hr.)
<i>Supply</i>		
(1) Imperial Dairy Institute Dairy	Below 500,000	Above 4 hr.
(2) Travelling milch cows	Below 500,000	Above 4 hr.
(3) Milk hawkers	Between 500,000 and 5 millions	Below 180 min. (3 hr.)
(4) Town dairies	Over one million (Maximum recorded over 90 millions)	Below 120 min. (2 hr.)

It will be seen from Table III that, amongst the samples of production milk obtained from individual animals on a modern dairy farm (the Imperial Dairy Institute) gave the lowest bacterial count and highest reduction time, and the herd milk and domestic cows' milk came next, followed by milk from town milk stables and village milk. On the supply side, milk from the dairy farm and travelling milch cow gave lower counts and higher reduction times than the supplies of milk hawkers and town dairies. It is obvious that the methods of production and handling obtaining at the different sources which have already been described have tended to give varying bacterial counts and reduction times. The very high counts of milk

from town stables and villages are evidently due to unclean methods. The same can be said of the milk hawkers and town dairies. The time elapsing between production and supply, unfavourable temperature conditions and contamination during haulage and distribution are the other contributing causes in these cases.

The methylene blue reduction test has received considerable attention overseas. It is now employed in the legal control of market milk in Great Britain. It is considered a simple, quick and cheap test and much work has been done on it to determine whether any correlation exists between the reduction time and plate count. In making this study the results given in Table I show that, while there

is a fair amount of general correlation between the plate counts and reduction times of the same samples of individual cows and buffaloes at the Imperial Dairy Institute, marked discrepancies occur in other cases. The latter

position is clearly brought out in Table IV in which, samples showing different plate counts have been arranged with their respective reduction times. It will be seen that, on the production side, out of the total of 662 samples

TABLE IV

Bacterial counts per ml. of milk.	Methylene blue reduction time in minutes						Total No. of Samples
	330 and over	329 to 240	239 to 180	179 to 120	119 to 60	Below 60	
<i>Production</i>							
500,000 and below	397 (59.97%)	262 (39.58%)	3 (0.45%)			...	662
200,001 to 500,000	5 (0.58%)	31 (40.70%)	28 (36.84%)	11 (14.47%)	1 (1.33%)	...	76
500,001 to 1 million	...	14 (13.86%)	45 (44.55%)	33 (32.67%)	9 (8.91%)	...	101
Over 1 million	...	10 (11.63%)	14 (16.28%)	27 (31.39%)	27 (31.39%)	8 (9.31%)	86
<i>Supply</i>							
200,000 and below	49 (35.25%)	90 (64.75%)	139
200,001 to 500,000	10 (12.19%)	65 (79.27%)	7 (8.54%)	82
500,000 to 1 million	...	3 (6.12%)	10 (20.41%)	21 (42.86%)	15 (30.61%)	...	49
Over 1 million	1 (0.69%)	18 (12.41%)	58 (40.00%)	68 (46.80%)	145

with plate counts of 200,000 and below, 397 (59.97 per cent.) gave reduction times of 330 minutes and over, 262 (39.58 per cent.) fell between 240 and 329 min. and 3 (0.45 per cent) between 180 and 239 min. On the supply side 139 samples gave plate counts of 200,000 and below, of which only 49 (35.25 per cent) had reduction times of 330 min. and over and 90 (64.75 per cent) fell between 240 and 329 min. Similar observations have been made by other workers. Malcolm and Reunick [1936] observed that a considerable proportion of milk samples which attained the Grade A* standard according to plate count reduced methylene blue within 5 hr. On the other hand many samples which could be classed as Grade A on the basis of reduction time fell out on account

of plate count. Thomas and Tudor [1937] also observed discrepancies. Nichols and Edwards [1936] found that samples giving counts of over one million per ml. reduced methylene blue within two hours. Recently, Kudelka [1941] observed a positive correlation; samples of milk showing 50,000 bacteria per ml. reduced the dye in about $5\frac{1}{2}$ hr., while samples containing over one million decolorized it in about 30 min. Those between these extremes gave intermediate reduction times. The results obtained in this work are different from the findings of Kudelka, but they fall in line with the observations of Nichols and Edwards, to some extent, as a majority (161 out of 231) of samples showing plate counts of over one million reduced methylene blue within two hours.

	Plate count per ml.	Methylene blue reduction time	
		Winter	Summer
Grade A* milk— Memo. 139/Foods [1937]	30,000 to 200,000	< $5\frac{1}{2}$ hr.	< $4\frac{1}{2}$ hr.
Grade A milk— Wilson, G. S. [1935]	30,000 to 200,000	< $6\frac{1}{2}$ hr.	< $5\frac{1}{2}$ hr.

From the above it will be evident that there is a lack of correlation between the plate counts and reduction times of the same samples but at the same time a general correlation is observed between plate counts of 200,000 and below and the reduction times of 240 min. (4 hr.) and over (Table IV). The fact that out of 801 samples which gave plate counts of 200,000 and below, only 446 (55.68 per cent) and reduction times of 330 min. ($5\frac{1}{2}$ hr.) and over while on the

other hand 798 (99.63 per cent) gave reduction times of 240 min. (4 hr.) and over supports this observation.

SUMMARY

(a) The bacteriological quality of milk produced and handled under different conditions in Bangalore has been studied by the application of methylene blue reduction test and plate count. A total of 1340 raw milk samples of

only morning production and supply has been examined. Samples of milk taken on production side were from (i) individual animals and herd (cow and buffalo) of the Imperial Dairy Institute Farm, (ii) town milk stables, (iii) domestic cows and (iv) village and those of

supply included, (i) Imperial Dairy Institute Dairy (cow and buffalo), (ii) travelling milch cows, (iii) milk hawkers and (iv) town dairies.

(b) A majority of the samples of milk from different sources showed the following bacterial counts and reduction times:

Source	Bacterial counts per ml.	Methylene blue reduction time
(a) Individual animals (Imperial Dairy Institute)	Below 50,000 . . .	Over 5½ hr.
(b) Herd (Imperial Dairy Institute and domestic cows)	Below 100,000 . . .	4 to 5½ hr.
(c) Dairy (Imperial Dairy Institute) and travelling cows	Below 500,000 . . .	Above 4 hr.
(d) Town stables	Between 200,000 and 5 millions.	2 to 5½ hr.
(e) Village	Between 500,000 and 10 millions	1 to 4 hr.
(f) Milk hawkers	Between 500,000 and 5 millions	Below 3 hr.
(g) Town dairies	Over one million (maximum recorded over 90 millions)	Below 2 hr.

(c) Although correlation is observed between plate counts and reduction times of the same samples of individual animals, marked discrepancies occur in other cases.

(d) In general, a better correlation is observed between reduction times of 4 hr. and above and plate counts of 200,000 per ml. and below, than between 5½ hr. and over and 200,000 and less.

(e) The bacteriological quality of milk from villages and town milk stables and of supplies made by milk hawkers and town dairies is unsatisfactory and improvements in the production, handling and distribution of milk, therefore, need urgent attention.

(f) The need for a properly organized and vigorous campaign on clean milk production and handling and an effective machinery for the

bacteriological control of market milk supplies is essential.

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A SMALL OUTBREAK OF STRANGLES IN ADULT PONIES

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AN infectious febrile disease occurred among the Institute transport ponies during the third week of June and lasted till the middle of October, 1941. Seven ponies were affected, of which three manifested high temperature, acute catarrhal symptoms and abscess formation of the submaxillary or parotid lymph glands; and the other four exhibited pyrexia and catarrhal symptoms associated with signs of pharyngitis and laryngitis. The ages of the affected animals were 4, 5, 5, 6, 12, 14 and 21 years. More or less common features were a rise in temperature to 103°–104° F. the presence of a nasal discharge at first serious and later mucopurulent, and that on attempting to

drink water much of it was returned through the nostrils. None of the animals was completely off-feed.

Reports were received that during this period a similar condition was prevailing among the Banjara-ponies in neighbouring areas around Bhowali and Naini Tal and that some of them died. It is quite likely that the infection was acquired at Bhowali, which was frequently visited by the ponies. All the affected Institute ponies, recovered with proper care and treatment.

ARTIOLOGY

Microscopical examination of nasal discharge and of pus from freshly-opened abscesses

showed short chains of streptococci. By cultural examination it was proved that five of the seven cases were associated with haemolytic streptococci which fermented salicin and trehalose, but not lactose and mannitol, thus resembling Edwards' Type B1 or Bazeley's Type 4.

TRANSMISSION EXPERIMENTS

1. With blood

Blood drawn at the height of temperature from three cases, Nos. 5, 6 and 7 of the series, two of which subsequently had parotid abscesses, were injected subcutaneously into a foal, No. 499, aged four months. Apart from a sudden and transient rise of temperature (to 103.4°F.) 14 days after the first injection, there were no symptoms.

Blood transmission was also carried out from the experimentally-infected foal with negative results, the blood at this time showing no bacteria in culture.

2. With culture

A 24-hour broth culture of streptococci, isolated during this outbreak, was sprayed into the nostrils of foal 499, 21 days after the last blood injection. Four days later, there was a definite rise in temperature associated with nasal discharge and swelling of the submaxillary region. This swelling was hard and painful and gradually spread over the face, especially on the left side. Metastatic abscesses then appeared over the submaxillary, parotid and pharyngeal regions, abscess formation being fully developed within nine days, when the temperature rose to 105.6° F. The abscesses were opened on that day and dressed antiseptically. It took nearly four weeks before the animal was declared cured.

IMMUNITY TEST

For this purpose cases 5, 6 and 7 and the artificially infected foal 499 were used. A 24-hour culture of streptococci, isolated during the outbreak was sprayed into the nostrils, a healthy foal No. 512 being included as control. During an observation period of three weeks, there was no sign of the disease in the recovered animals, while the control foal developed from the 3rd day a temperature which gradually rose to 104.6° F. on the 7th day and persisted for a week, when thick creamy discharge from the right nostril was present. This discharge on microscopical and cultural examination yielded haemolytic streptococci. The control foal was left untreated but developed no further symptoms.

From the above observations, it is seen that the incubation period in the two artificially-infected animals (foals 499 and 512) was three to four days. The immunity test revealed that animals which had once suffered from the disease, naturally or artificially acquired, attained a strong immunity lasting for at least four months. To test the duration of immunity, the same strains of streptococci maintained for a year in culture media were used on healthy as well as on recovered ponies. None of these animals got the disease, indicating that the bacteria had lost its virulence. An attempt at exalting the virulence by rabbit passage was unsuccessful.

DISCUSSION

In this small outbreak only adult animals were at risk, but in three of the seven affected the lesions were those of typical strangles. In this connection it may be noted that the ponies had lived a relatively isolated existence in the Kummaun hills and had only rarely been in close contact with other equines. Their susceptibility would therefore be high. Experimentally, it is interesting to note that a clinical attack of strangles was readily set up in foals by means of a streptococcus culture. Bazeley [1943] found that positive results could only be obtained with certainty by using a very young (4½ hr.) culture, but in this work there was no difficulty in setting up the disease in young foals with a 24-hour culture of an early generation.

SUMMARY

1. An account is given of a small outbreak of strangles in adult ponies, associated with haemolytic streptococci of Edwards' type B₁ or Bazeley's equine type 4.

2. Blood of affected animals taken at the height of fever was non-infective, but strangles in its typical form was easily set up experimentally in foals by nasal installation of a 24-hour broth culture of the streptococcus.

3. Naturally and experimentally infected animals were proved to be immune.

ACKNOWLEDGEMENT

The authors acknowledge their thanks to Mr. J. R. Haddow for encouragement and to Mr. V. R. Rajagopalan, for assistance on the bacteriological side.

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THE COMPOSITION OF PRE-MILK AND COLOSTRUM OF DAIRY COWS OF THE PEDIGREE SAHIWAL HERD AT THE IMPERIAL AGRICULTURAL RESEARCH INSTITUTE, NEW DELHI*

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(Received for publication on 1 November 1943)

ONE of the practices in the management of the Sahiwal Herd at this Institute is to premilk heifers and cows about to calve. It is usually done for a period of 5 to 15 days before the animals are due to calve. The milk thus obtained is called premilk; it is yellowish in colour and thick. It is not mixed with the normal milk, but is used in the feeding of calves.

Two or three instances occurred in which heifers began to develop udders and to yield

milk, even before they were put to the bull. The quantity of the premilk ranged from 5 to 15 lbs. per day. An instance is that of heifer No. 769 which was born on 18th March 1936 and was put to the bull on 29th June 1938. This heifer was giving milk even before she was put to the bull. The milk resembled normal milk in physical appearance. In chemical composition also it was similar to normal milk as can be seen from the analytical data given in Table I.

TABLE I

	Heifer No. 769		Heifer No. 776				
	13th July	14th July	10th June	15th June	20th June	13th July	14th July
Total solids	14.0	14.2	14.1	14.7	14.0	14.9	14.5
Fat	4.8	5.0	4.3	5.1	4.5	5.0	5.1
Acidity	0.13	0.13	0.20	0.21	0.17	0.19	0.18
Lactose	5.1	4.6	5.1	5.2	5.2	5.4	5.0
Total Protein	3.4	3.4	3.2	3.1	3.1	3.0	3.1
Casein	2.7	2.7	2.8	2.6	2.6	2.5	2.6
Albumin and Globulin	0.7	0.7	0.4	0.5	0.5	0.5	0.5
Coagulation test	no	no	no	no	no	no	no
Ash	0.67	0.67
Sp. gravity	1.029	1.029

There was another heifer (No. 776) which was born on 10th June 1936 and was put to the bull on 25th December 1937. This heifer began to give from the 5th or the 6th month of gestation secretions which were in all appearance like normal milk and analysed like normal milk, as can be seen from the results given in Table I above.

These milks were submitted to nutrition tests by Dr R. K. Pal, a medically qualified and experienced officer stationed at the Institute as Liaison Officer between Human Nutrition and Agricultural Research. He carried out experiments with rats and a large number of boys in the Estate and found that this abnormal milk was in no way deficient in composition and nutritive value.

It was, therefore, of interest to ascertain the chemical composition of the premilk i.e., milk obtained a few days before calving, as a preli-

minary to the starting of further investigations.

From the available literature on the subject, it is seen that the investigations were along two main lines—(a) From secretions before calving to colostrum, studied by Woodman and Hammond [1923] and Ashdell [1925] and (b) From colostrum to normal milk, studied by Bergmann and Turner [1937], Dover and Sivasubramaniam [1938] and Shah [1936].

In this investigation we have observed the variation in the composition of premilk of heifers and cows of different calving and ages up to the day of parturition and continuing to the state of normal milk secretion. The secretions were withdrawn twice daily; only morning secretions were analysed.

*The material for this article was taken from the thesis submitted by S. K. Mukherjee in 1938 for the Associateship of the Imperial Agricultural Research Institute.

EXPERIMENTAL

The analyses were carried out during the period 10 June, 1938 to 21 September, 1938.

Samples of milk were received from cows of the pedigree herd of Sahiwal of the Institute. Particulars of the animals whose secretions were analysed are given in Table II.

TABLE II
Description, etc. of the animals

No.	Date of birth	Date of service	Period of gestation at the time of analysis		Calved after service	
			Months	Days	Months	Days
776. Heifer	10-6-36	25-12-37	6	15
769. Heifer	18-3-36	20-6-38	..	15
771. Heifer	6-4-36	18-11-37	9	6	9	9
772. Heifer	29-4-36	3-12-37	9	3	9	8
773. Heifer	3-5-36	25-11-36	9	9	9	10
763. Heifer	29-12-35	11-9-37	9	2	9	17
736. Cow 2nd Calver	1-12-34	25-11-37	9	14	9	16
740. Cow 2nd Calver	27-12-34
742. Cow 2nd Calver	5-1-35	7-11-37	9	7	9	16
716. Cow 3rd Calver	14-3-34	25-11-37	9	4	9	6
710. Cow 3rd Calver	12-2-34	22-11-37	9	9	9	12
674. Cow 3rd Calver	10-12-32	24-11-37	9	12	9	15
706. Cow 3rd Calver	23-12-33	26-9-37	9	6	9	9
644. Cow 4th Calver	10-9-31
653. Cow 4th Calver	..	23-11-37	9	6	9	7
604. Cow 5th Calver	..	5-12-37	9	4	9	4
569. Cow 8th Calver	..	25-11-37	9	15	9	15

The samples of secretions were obtained in clean bottles and were kept in iced water at a temperature of about 10° C. They were immediately taken to the laboratory for analysis and were shaken properly before each pipetting.

Total solids, ash, casein, lactose and acidity were estimated according to the A. O. A. C. methods of analysis [1935]. Fat was estimated by Gerber's H_2SO_4 -amylalcohol method and Sp. gravity by means of the Westphal balance (7). Total protein was separated by 4 per cent. trichloroacetic acid from the non-proteins (3). Albumin was at first determined directly by the A. O. A. C. method [1935], but it was subsequently found not suitable in presence of globulin. Later on, globulin was directly estimated by the satd. $MgSO_4$ method [1937], and the albumin content was obtained by difference.

Colostrum milk and premilk coagulated on heating. The coagulation was studied as follows—colostrum or premilk was diluted with the requisite amounts of normal milk in test tubes to make dilutions of 10 per cent., 15 per cent., 20 per cent., and so on with respect to normal milk. The test tubes were then stoppered and kept in a boiling water bath. After an hour or so, flocculation, if any, was observed.

The minimum dilution for complete coagulation, was put down as the coagulating power of the secretion. Thus 10 per cent coagulation means that 1 cc. of colostrum is the minimum quantity coagulating 9 cc. of normal milk. Since normal milk does not coagulate by itself the coagulating test was a criterion for judging the stage of transition from colostrum to normal milk.

The results of analysis are summarized in Tables III to VI. Instead of showing the day to day variations of the various constituents, only the compositions of the secretions on the first day of withdrawal, just before calving and just after it, and at the normal milk stage are recorded. These clearly indicate the transitional phases in the production of milk.

The change from premilk to normal milk is characterized by two transitional stages. Firstly, premilk to colostrum and secondly from colostrum to normal milk. The compositions of the secretions with reference to these two stages are discussed below.

Premilk and colostrum have higher percentages of total solids than normal milk. In premilk the high total solids are associated with a high protein content, whereas in colostrum it is due to the high fat content.

TABLE IV

Cow 2nd calver No.	No. of days of pre-milking	Before calving		After calving		Before calving		After calving	
		Initial analysis	Just before calving	Just after calving	Normal milk	Initial analysis	Just before calving	Just before calving	Normal milk

*Variation in total solids**Variation in sp. gravity*

736	2	15.8	14.8	16.4	15.2	1.033	1.031	1.032	..
740	9	16.5	14.3	22.1	17.8
742	9	18.7	20.7	25.7	16.8

*Fat**Lactose*

736	2	5.4	3.9	5.8	5.0	2.8	4.1	3.5	4.3
740	9	1.1	1.6	5.9	8.1	2.6	3.1	3.4	4.1
742	9	0.3	11.0	13.0	7.1	1.7	3.8	3.3	3.8

*Acidity**Ash*

736	2	0.29	0.28	0.28	0.27	0.72	0.87	0.74	0.67
740	9	0.39	0.38	0.43	0.35
742	9	0.23	0.22	0.22	0.23	0.72	0.81	0.82	0.76

*Total protein**Casein*

736	2	4.0	5.3	4.2	3.8	2.8	2.8	2.7	3.3
740	9	11.0	6.4	9.6	3.9	2.9	2.5	3.7	3.1
742	9	13.1	3.8	3.9	3.9	3.3	3.0	3.0	3.2

*Albumin**Globulin*

736	2	0.56	..	0.64	0.14	0.68	0.56	0.86	0.35
740	9
742	9	3.3	0.70	0.90	0.62	6.6	0.01	0.03	0.14

*Albumin and globulin**Coagulation test*

736	2	1.2	..	1.5	0.49	no	no	50	no
740	9	8.1	3.9	3.9	0.80	..	30	10	no
742	9	9.9	0.71	0.93	0.76	..	90	80	no

TABLE V

Cow 3rd calver No.	No. of days of pre- milking	Before calving		After calving		Before calving		After calving	
		Initial analysis	Just before calving	Just after calving	Normal milk	Initial analysis	Just before calving	Just after calving	Normal milk
Variation in total solids									
674	4	17.4	17.9	24.0	15.7	1.046	1.040	1.050	..
706	3	15.3	18.9	13.3
710	3	14.0	21.0	15.0	15.0	1.038	1.029	1.023	1.023
716	2	17.0	26.0	17.2	17.2	1.039	1.043	1.031	1.030
Variation in sp. gravity									
674	4	1.4	1.7	7.8	7.1	2.3	3.5	2.8	3.9
706	3	0.5	0.4	7.2	3.8	2.5	3.1	3.2	4.3
710	3	0.8	4.3	11.0	3.3	2.3	3.3	3.3	3.4
716	2	3.2	3.2	11.7	6.6	2.0	3.3	3.7	3.8
Fat									
674	4	1.4	1.7	7.8	7.1	2.3	3.5	2.8	3.9
706	3	0.5	0.4	7.2	3.8	2.5	3.1	3.2	4.3
710	3	0.8	4.3	11.0	3.3	2.3	3.3	3.3	3.4
716	2	3.2	3.2	11.7	6.6	2.0	3.3	3.7	3.8
Lactose									
674	4	0.30	0.28	0.42	0.26	0.74	0.79	0.77	0.68
706	3	0.27	0.27	0.33	0.26
710	3	0.28	0.24	0.23	0.16	0.87	0.78	0.70	0.83
716	2	0.34	0.39	0.42	0.30	0.70	0.89	0.73	0.74
Acidity									
674	4	0.30	0.28	0.42	0.26	0.74	0.79	0.77	0.68
706	3	0.27	0.27	0.33	0.26
710	3	0.28	0.24	0.23	0.16	0.87	0.78	0.70	0.83
716	2	0.34	0.39	0.42	0.30	0.70	0.89	0.73	0.74
Ash									
Total protein									
674	4	10.2	6.4	11.1	3.5	3.4	3.0	3.5	2.7
706	3	9.7	7.2	8.1	3.7	2.9	2.9	3.5	2.9
710	3	7.4	4.3	4.3	3.8	3.1	2.9	2.9	3.0
716	2	7.7	6.9	6.9	4.6	4.8	4.6	4.2	3.7
Casein									
674	4	1.6	0.72	4.2	0.78	5.1	2.7	3.4	Nil
706	3
710	3	1.0	0.41	0.33	0.28	3.3	0.99	0.99	0.52
716	2	1.3	0.63	0.53	0.62	1.7	1.7	2.2	0.27
Albumin									
674	4	1.6	0.72	4.2	0.78	5.1	2.7	3.4	Nil
706	3
710	3	1.0	0.41	0.33	0.28	3.3	0.99	0.99	0.52
716	2	1.3	0.63	0.53	0.62	1.7	1.7	2.2	0.27
Globulin									
Albumin and globulin									
674	4	6.7	3.4	7.6	7.78	10	20	10	no
706	3	6.8	4.3	4.6	0.80	20	30	20	no
710	3	4.3	1.4	1.3	0.80	10	60	60	no
716	2	3.0	2.3	2.7	0.89	10	20	20	no
Coagulation test									

TABLE VI

Cow 4th calver No.	No. of days of pre-milking	Before calving		After calving		Before calving		After calving	
		Initial analysis	Just before calving	Just after calving	Normal milk	Initial analysis	Just before calving	Just after calving	Normal milk
Variation in total solids									
644	..	20.0	22.0	21.4	21.3	1.050	1.040	1.040	1.030
653	..	17.7	..	18.7	17.5
Fat									
644	..	0.2	0.7	11.4	8.8	..	3.5	3.2	3.9
653	..	0.5	..	4.6	7.4	1.8	..	3.5	3.9
Lactose									
Acidity									
644	..	0.41	0.33	0.35	0.32	..	0.85	0.63	0.85
653	..	0.25	..	0.33	0.23	1.0	..	0.52	0.67
Ash									
Total protein									
644	..	15.0	5.0	4.8	4.2	3.2	3.7	3.2	3.6
653	..	12.7	..	7.9	3.9	3.1	..	3.5	3.3
Casein									
Albumin									
644	0.58	7.2	..	4.3	0.7
653	..	2.4
Globulin									
Albumin and globulin									
644	..	11.8	2.3	1.6	0.60	10	40	40	no
653	..	9.6	0.65	20	..	30	no
Coagulation test									

normal milk. This decrease is due to the reduction of two of the proteins, viz., albumin and globulin, and especially of the latter. Only in some cases the protein content shows an abrupt rise immediately after calving, but this is far from a general occurrence. In cases where premilking has been done the protein content scarcely shows increase but where premilking has not been done at all, or only two or three days before parturition, the protein content is usually high but shows no further rise (see below).

Casein content does not vary much for the different animals and shows no transitional change. Even when the total protein is high the casein content remains almost unaltered.

Globulin content remains at a high level in premilk; it then gradually diminishes. Occasionally, a rise in the globulin content may be observed in the colostrum, but if premilking is done for a long time the chance of such a rise is negligible. When no secretion is drawn before calving the globulin content of colostrum is considerably high, and abruptly drops to a

low value on the day following calving. In such cases the total solids, fat, specific gravity and acidity may also be high. Two such cases are enumerated below in Table VII.

TABLE VII.

	5th Calver No. 6048th Calver No. 569			
	Immediately after calving	Normal milk	Immediately after calving	Normal milk
Total solids	15.7	16.2	31.5	18.4
Fat	10.2	10.5	3.5	9.1
Acidity	0.58	0.28	0.45	0.26
Lactose	1.8	4.3	2.4	4.1
T. Protein	19.3	4.3	9.8	3.9
Casein	4.3	3.6	3.5	3.2
Albumin	2.0	0.10	2.2	0.63
Globulin	13.0	0.58	4.0	0.03
Coagulation test.	10	no	30"	no
Ash	0.95	0.79	0.76	0.66
Sp. gravity	1.08	..	1.06	..

Ash content varies slightly for the different cows. It is only somewhat higher in colostrum and premilk than in ordinary milk.

The specific gravity of premilk and colostrum is generally somewhat higher than that of normal milk. These high values are parallel with the high viscosity of the secretions.

Variations of the different constituents from animal to animal are better correlated with the

period of "drying" than with the age of the animals or other characteristics. The range of variations of some important constituents is given in Table VIII from which it would appear that the range is not much different for the different animals but the absolute values are dependent upon the length of time the animals have been kept "dry". The length of the drying period has been calculated from Table II as the difference between the figures given in columns 5 and 6.

TABLE VIII

		Premilk (Range)	Colostrum (Range)	Normal milk (Range)	Length of drying period (Range) in days
Total solids	Heifers	13.5—24.3	18.4—34.8	13.1—18.5	1—15
	Cow, 2nd calvers	15.8—18.7	16.4—25.7	15.2—17.8	2—9
	Cow, 3rd calvers	13.7—17.4	18.9—26.0	13.3—17.2	2—3
Fat	Heifers	0.9—3.2	5.0—9.5	5.2—7.3	
	Cow, 2nd calvers	0.3—6.4	5.8—13.0	5.0—8.1	
	Cow, 3rd calvers	0.5—3.2	7.2—11.7	3.3—7.1	
Total Protein	Heifers	3.7—16.0	3.5—8.9	3.5—4.0	
	Cow, 2nd calvers	4.0—13.1	3.9—9.6	3.8—3.9	
	Cow, 3rd calvers	7.7—10.2	4.3—11.1	3.5—4.6	
Globulin	Heifers	0.0—6.7	0.83—2.7	0.04—0.39	
	Cow, 2nd calvers	0.68—6.6	0.03—0.86	0.14—0.35	
	Cow, 3rd calvers	1.7—5.1	0.90—3.4	0.27—0.52	

From the results of analysis (Tables III, IV, V and VI) summarized above, it would appear that in general the secretion withdrawn before parturition, i.e., the premilk, is characterized by (1) a viscous consistency, (2) high total solids, (3) high protein content, especially globulin, (4) high acidity, (5) low lactose content and (6) low fat content. If the premilking period is long the percentages of the constituents tend to equalize with those of normal milk, excepting that the fat content shows an abrupt rise on the day of calving or the following day, there being hardly any increase in the globulin or the albumin content. If, however, the period of premilking is short (e.g., two or three days only) the transition from premilk to colostrum is more abrupt. The withdrawal of premilk has thus a normalizing effect on the composition of colostrum (cf. also results in Table V).

SUMMARY AND CONCLUSIONS

The secretions from the udders of a number of heifers and cows of different calvings and ages, withdrawn before calving, after calving, and up to the normal milk stage, were analysed. Total solids, fat, proteins, especially globulin,

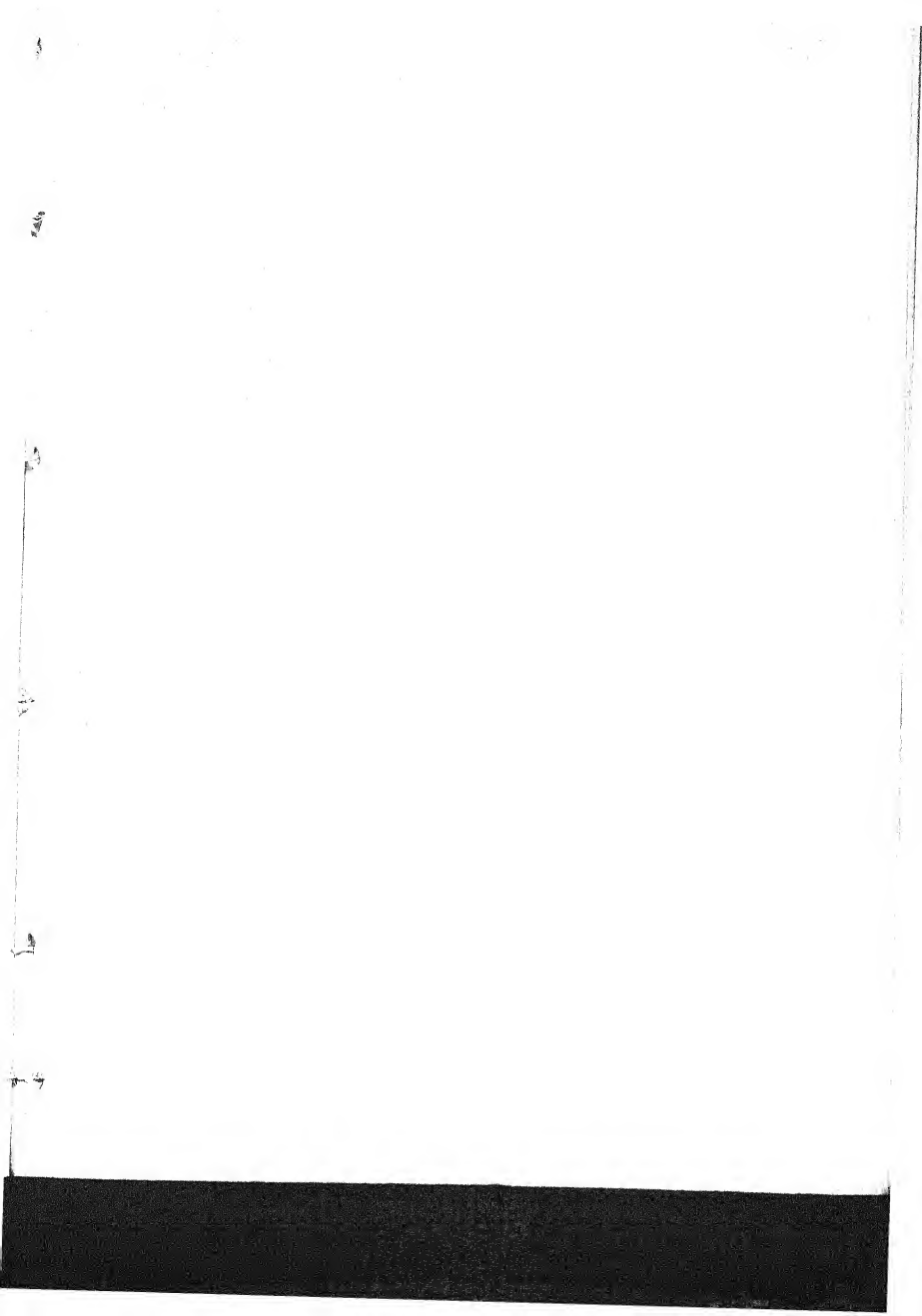
showed characteristic variations which could be correlated with the transitional phases from premilk to colostrum and from colostrum to normal milk.

Two heifers yielded large quantities of secretions which were almost identical with normal milk in both physical appearance and chemical composition. When premilking is done for a long time the composition of the secretions tends to become similar to that of normal milk.

We take the opportunity to express our thanks to Mr. Wynne Sayer, the Imperial Agriculturist and to Mr. Fernandez, the Cattle Superintendent for the supply of samples of milk for the investigation.

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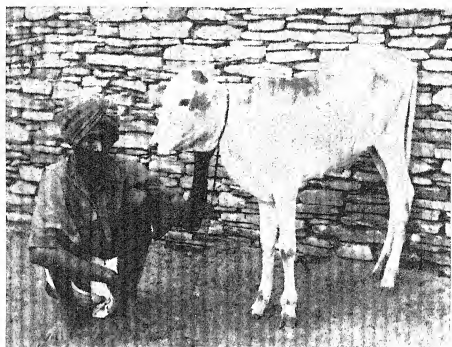


FIG. 1. An affected calf showing exostosis of the left fetlock and the knee joints. Rough coat also seen with atrophy of the intercostals beginning.



FIG. 2. An affected bullock showing the callus formation on the rib and also intercostal muscles atrophy.

FLUOROSIS OF CATTLE IN THE MADRAS PRESIDENCY

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(Received for publication on 26 March 1943)

(With Plate XXII)

IN 1934-35 the writer recorded the existence of a disease which on clinical ground he described as chronic rheumatic arthritis in cattle in certain villages of Kurnool district. Since then the condition has been reported from other districts and the present article includes an account of the disease as observed in cattle in the Madras Presidency.

The disease is locally known in Telugu districts as *Voyupotlu* or *Voyunoppulu* (*Voyu*=Rheumatism, *Potlu* or *Noppulu*=pains) and in Tamil as *Keelooyu* (*Keel*=joint and *Voyu*=Rheumatism).

OCCURENCE AND SYMPTOMS

This disease occurs in several villages of various taluks of Kurnool, Anantapur, Cuddapah and Nellore districts, the endemic regions being adjacent to Hyderabad State where this disease is also prevalent. The endemic areas of the Madras Presidency lie on the borders of the Nallamalais range, situated on one side of the Kurnool district and divided by the Thungabhadra and Kistna rivers.

The disease generally affects breeding bulls, working bullocks and cows. Calves and buffaloes suffer only occasionally (Plate XXII, fig. 1). Few cases have been observed in sheep, but none in other domestic animals.

Amongst cattle the condition has been observed more frequently in Ongole breed of cows in milk and bullocks at work kept under stall-feeding condition. Number of cases have been observed in cows during lactation period and after their first calving. Only two cases have been observed in sheep, both of which were handfed.

Animals of the Ongole breed appear to be more susceptible than others; Mysore, Alambadi, and in certain villages 5 to 10 per cent of cattle of this breed might be found affected.

The conditions is rare in young animals and a few cases only have been recorded in suckling calves.

Cases of the disease may be observed throughout the year, but the symptoms appear to be more marked during the ploughing season and at the commencement of rains.

The history given by the owners is that the animals purchased when young, get the affection

one or two years after their arrival in the endemic area.

The first indication of this condition is lameness in one or more limbs, more often the forelimbs, and more particularly in the shoulders, which appear to be thrown forward. The lameness is usually a constant feature, generally obscure and progressive in nature and metastatic in character.

In about six months to a year from the onset of lameness, the animal shows stiffness of gait, evinces pain while walking, and finds difficulty in arching its back.

Later on the animal shows extreme emaciation due to atrophy of muscles all over the body, experiences difficulty in lying down and getting up and finally remains recumbent.

The hoofs are found twisted, distorted and over grown, the digits having a tendency to separate, and the points of the toes frequently curving inwards.

Skeletal abnormalities are manifested by swollen joints and exostoses over the long bones and the jaw. These appear as hard, bony enlargements on the medial and lateral aspects of the joints and the bones giving them a thickened or deformed appearance. In very severe cases, the pasterns becomes affected with even ankylosis of the joints so that the limbs present a club-shaped appearance, causing marked lameness and a peculiarly stiff gait.

Calluses are noticed on the prominent bones and the ribs. These occur in the form of nodular elevations on the middle of the ribs in a lengthwise manner on one or both sides and range in size from a walnut to a hen's egg.

The animal feels pain over the exostosis.

In certain endemic areas the affected animals show discoloration of teeth, with brownish pigmentation and mottling, and even chipping off at places.

In the early stage of the disease animals feed normally, but later the appetite becomes impaired, coat becomes harsh and rugged, emaciation advances and death ensues.

In young stock, growth remains poor in spite of good feeding.

Other symptoms include lowered fertility, delayed oestrus, sterility reduction in milk yield

and in some instances photophobia. The course of this disease runs from a few months to a few years, but in buffaloes the duration is shorter.

POST MORTEM FINDINGS

Due to religious sentiments of the people the writer had some difficulty in getting affected animals for destruction and *post mortem* examination. An opportunity was, however, availed for examining *post mortem* of two animals destroyed in *extremis* in a very badly affected village. In both, the internal organs appeared to be normal and the lesions were confined to the skeleton only. The following is the protocol of *post mortem* findings.

Case No. 1. The hip and shoulder joints were severely inflamed, metatarsal bone and the knee joints showed bony exostosis and diffuse periostitis and the nodular prominences present on the ribs were porous in character. The median suture in the skull showed a tendency to give way and the mandibles of the lower jaw were disunited at the symphysis. The epiphysis of the left humerus had the tendency to separate from the proximal end and those of the radius and ulna at their distal ends. The cancellated tissue in the interior of the long bones was found infiltrated with a lardaceous material.

Case No. 2. Of the ribs examined, one showed partial fracture about its middle at two places and another showed the two fractured ends, one riding over the other with callus formation around them. None of the long bones showed any exostosis, but numerous osteophytes were found on the non-articular surface of one of the os pedis bones. The cancellated tissue of the long bones showed infiltration with lardaceous material.

ETIOLOGY AND EPIZOOTIOLOGY

In this rheumatic affection of cattle, the etiological factors likely to be considered are (1) in balance of diet with regards to its mineral contents (2) deficiency of phosphorus (3) deficiency of vitamins and (4) fluoride poisoning.

From investigations carried out by the Government Agricultural Chemist, Coimbatore on samples of pasture grass from affected and healthy areas, it has been shown [Ramiah, 1941] that the former contains a high Ca and low P and the latter normal values for these minerals. Similarly, 14 samples of other fodders sent to the same worker for analysis revealed a striking imbalance with regard to these minerals. Again, a survey of pastures of Malabar and Kurnool districts carried out by

the Government Agricultural Chemist showed a shortage, if not the actual deficiency of these minerals. In the former district both CaO and P_2O_5 were low, whereas in the latter a shortage of P_2O_5 only was revealed.

The deficiency of P in the pasture leads to lowering of inorganic P value of blood, and in order to obtain further evidence regarding the etiological significance of this factor, blood samples from affected and normal animals were analysed by the Government Agricultural Chemist, Coimbatore with the result that whereas in the latter Ca and P both showed a normal value, in the former, P content was found to be low. In certain number of cases the P value was found to be below 1 mg. per 100 c.c. of blood. Blood samples sent from Madras cases for analysis at the Imperial Veterinary Research Institute, Izatnagar, showed no variation from normal; but those sent from Hyderabad, where a similar disease occurs, proved deficient in P.

Vitamin A deficiency was suspected to play a part in causation of this disease as the number of cases are less in the rainy season when the animals usually ingest plenty of green fodder.

Analysis of well water and soil samples from certain affected localities have revealed the presence of fluorine in a concentration higher than the amount present in those of healthy areas. Representative rocks and soils of an area stretching from Kurnool division to Markapur revealed on analysis a high content of fluorine. According to the Govt. Agricultural Chemist, Coimbatore [Ramiah, 1938-39] the Cuddapah-Kurnool system of sedimentary rocks shows a high fluorine content, ranging up to 960 part per million or about 500 times that of well water. Work carried out at the King Institute of Preventive Medicine, Guindy, show that the highest fluorine concentration is to be found to lie in the narrow belt of granitoid rocks stretching north, north-east from south of Kanigiri, Nellore and to the Krishna river near Annawati in Guntur. The presence of fluorides in high toxic quantities in certain areas, especially North Nellore and isolated places in Cuddapah and Anantpur has also been noticed.

Analysis of well water samples by the Govt. Agricultural Chemist [Ramiah, 1938-39] reveals a fluorine content ranging from 0.5 to 2 parts per million in samples from the affected areas, and a little or none from those of healthy ones. Table I shows the fluorine content of water samples collected from various Talukas in four endemic areas of the Presidency:

TABLE I
The fluorine content of water samples

No.	Talukus	Flouride content in sample				Total
		Nil or trace	Under 1 p.p.m.	1 to 3 p.p.m.	Over 3 p.p.m.	
I. Kurnool District						
1	Koilkuntla	6	23	25	1	55
2	Sirvel	..	10	8	1	19
3	Cumbum	..	7	7	..	14
4	Markapur	1	2	4	..	7
5	Dhono	..	1	1	..	2
6	Kurnool	..	8	1	..	9
7	Nandyal	..	2	2
II. Nellore District						
1	Podili	..	2	12	3	17
2	Darsi	..	1	8	7	16
3	Kanigiri	1	..	1
4	Gudur	1	..	1
III. Anantapur District						
1	Anantapur	..	1	1	..	2
2	Kalyandrug	1	1	2
IV. Guntur District						
1	Vinukonda	..	1	1	..	2
Total		8	59	70	12	149

The table shows that nearly 58 per cent of the affected villages contain one to three parts or more of fluorine per million. In one village of Nellore district the stream water was found to contain 13.85 p. p. m. of fluorine. The analysis of water from certain 'curative' villages has revealed a fluorine content less than 0.5 p. p. m.

Increased diuresis and pronounced pathological changes in kidney suggested that the high concentration of fluorine ingested with water had some deleterious effect on this organ when passed through urine, but twelve samples of urine sent for the detection of fluorine at the Imperial Veterinary Research Institute, Izatnagar, revealed nothing of significance. Samples of teeth from the affected animals were analysed at this Institute with the results quoted below.

The material forwarded by you has been analysed for their fluorine content which appears to be somewhat higher than that in normal calves as reported by some Western workers.

Field investigations reveal that there might be several factors, all exercising their effects on the etiology, occurrence and the progress of the disease. The endemic areas are known to be related to certain type of rock formation imparting to the soil, water and the herbage a

higher concentration of fluorine and a state of unbalance with regard to Ca and P. When the affected animals are removed to certain 'curative' villages where these soil and herbage abnormalities do not exist, they develop freedom from the disease.

Although it is not unlikely that the animals in these localities suffer from a mild form of hypophosphorosis, certain epizootological factors suggest that the condition described in this paper may be looked upon as fluorine intoxication. These are (1) spontaneous cure of clinical cases when removed to certain 'curative' villages, where the soil, herbage and water contains a normal amount of fluorine, (2) the presence of mottling and brown pigmentation together with the chipping tendency of enamel of teeth. This feature is characteristic of human fluorosis, (3) in cattle enzootic areas of this disease in the Presidency human beings are also known to suffer from skeletal and dental abnormalities as described by Shroff *et al* [1937].

TREATMENT

So far any form of treatment has not been found effective in advanced cases of the disease although cases in early stage are amenable to treatment. Mention has already been made of certain 'curative' villages where the general practices are paddy cultivation, irrigation from tanks and less ground-nut cultivation. Animals, when taken to these villages during early stage of the disease, show gradual improvement without any treatment. This is attributed to a change over from a brackish water to rain or soft water of these villages.

(a) *Curative.* During the year 1934-35, Mahajan, in Hyderabad, treated some cases with sterilized bone meals and reports encouraging results as no further cases occurred when he visited the same tract again after five months. He advocated this treatment both as curative and preventive. In this Presidency, bone-meal was first tried in Joladarsi village of Koilkuntla Taluk in Kurnool district in the year 1934-35. The affected animals were given each one ounce of bone-meal per day for six months and complete recovery was observed in two animals thus treated. On another occasion, seven animals in Cuddapah and thirteen in Kurnool district were treated with bone-meal given in doses of two ounces daily to each animal. As a result of this treatment in about two months from the administration of bone-meal, the animals in the early stage of the disease showed rapid improvement. In well-advanced cases with marked anatomical

abnormalities such as bony exostoses etc. the bone-meal feeding relieved them of acute symptoms like lameness and painful joints, but had a very little effect in reducing the exostoses. The duration of the treatment is long and marked improvement is noticed after a course of treatment lasting from six months to one or two years. When the treatment with bone-meal is discontinued on finding improvement in condition, there is a possibility of relapse, hence affected animals in enzootic areas require treatment as long as they live.

(b) *Preventive.* Prophylactically the bone-meal may be recommended for supplementing the diet of cattle grazing on deficient pasture, as it would not only prevent the disease, but also improve the rate of growth of young stock. Moreover the bone-meal fed animals have got a tendency to maintain the advantage during the seasons when the grazing is generally poor. The Government Agricultural Chemist recommends the addition of lime to the water of the wells in affected localities, as this process reduces the fluorine content of the water.

SUMMARY

1. This disease in cattle known as *Voyo potlu* locally, was first observed in Madras Presidency in 1934-35 in Kurnool district. Since then its incidence has been known in certain villages of Nellore and Cuddapah districts around the hills, being related to the condition of the soil, pasture, foodstuffs and water and the nature of the prevailing agricultural practices.

2. There is no seasonal incidence. The disease runs a chronic course affecting 5 to 10 per cent of cattle population in an enzootic area. One peculiarity about the disease is that while its incidence is heavy in one village, the neighbouring village may be free from it.

3. The main etiological factor is the presence of fluorine in an abnormal amount in soil.

pastures and the water, followed by aphosphorosis and vitamin deficiency.

4. The main feature of the disease is the presence of exostosis on the ribs and the joints, and overgrowth and deformity of hoofs. Fractures and ulcerations of articular heads and a few bony prominences are the characteristic lesions on *post mortem* examination.

5. There is no complete or successful treatment in advanced cases. In early stages treatment is only palliative.

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PROTEINS FOR EGG PRODUCTION, II

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PREVIOUS experiments carried out by Macdonald and Bose [1943] demonstrated that a cereal ration containing 10.4 per cent protein gave poor egg production, even when supplemented with common salt and liberal amounts of green food and calcium. The addition of vegetable proteins, such as soya bean meal and earlnut

meal, to the cereal rations had no beneficial effect, but supplements of separated milk and meat offal on the other hand markedly improved egg production. In view of the importance of these results another experiment was designed to throw more light on several other aspects of the work. In this experiment all the birds

were fed wet on mash, whereas in the previous experiment all the groups, except that on meat offal, were fed on dry mash. Comparisons were made between separated milk and meat offal supplements (intestines of animals from the slaughter house) and low protein cereal diets with and without salt supplements.

EXPERIMENTAL

Stock. A total of 200 six to seven month old White Leghorn X Rhode Island Red pullets were divided into four comparable groups and fed on the experimental rations for a period of 280 days. The groups were housed under similar conditions and had access to large grass runs. All the birds were trapnetted throughout the experiment.

Feeding. The four groups were fed on mash and grain rations. The basal mash fed to all groups consisted of wheat bran 50 parts, yellow maize meal 30 parts and ground oats 20 parts. The grain ration consisted of a mixture of equal parts of yellow cracked maize, wheat and paddy. Broken limestone and water were fed *ad libitum*, together with liberal amounts of succulent green food.

Group I (Basal Group) was fed the basal diet only. Group II (Salt Group) received the basal diet *plus* 1 per cent common salt in the mash. Group III (Milk Group) received the basal mash supplemented with 0.5 per cent common salt and approximately 5 oz. of separated milk per bird per day. Group IV (Meat offal Group) received the basal mash supplemented with 1 per cent common salt and 1.2 oz. of meat offal per bird per day.

All four groups received wet mash according to appetite at 7.30 A.M. The amount fed varied according to appetite, the usual amount being about 1.2 oz. of dry mash per bird. The mash was fed in a crumbly state, and the amount fed was limited to what the birds would readily consume in 10 to 15 minutes. The wet mash for Groups I and II was made up by adding water only. The mash for Group III was mixed with separated milk instead of water and the balance of the milk ration for the day was fed *ad libitum* in a top-filling fountain. Group IV received the mash mixed with meat offal. The meat offal required for the group was weighed out and cooked in a small quantity of water for a period of one hour. The offal was then run through a mincing machine and mixed with the mash. The residual water from the cooking was also mixed into the mash.

About 0.5 oz. of grain per bird was fed to each of the groups at 10 A.M. Dry mash was also

given in waste-proof hoppers from 12 noon until 4 P.M. Grain according to appetite (approximately $1\frac{1}{2}$ oz. per bird) was given to each of the groups at 5 P.M. All the four groups received water to drink but Group IV also received the balance of their separated milk ration in drinking vessels. In order to ensure a complete consumption of the milk, the water vessel was removed from the milk group at the time of feeding the milk and replaced when the milk was finished.

All the foods consumed were regularly analysed, and every effort was made to keep the protein percentages in Groups III and IV at a constant level. Over the whole experiment, the average percentage protein in the basal mash was 10.4. The corresponding figure for the grain mixture was 9.7 per cent. The separated milk contained 9.7 per cent dry matter and 3.5 per cent protein. The corresponding figures for the meat offal were 18.0 and 12.2.

Egg production. Table I gives the average egg production per bird for each group for each period of four weeks and the average egg production per bird for the whole period.

TABLE I
Average egg production per bird

Period	Groups			
	I	II	III	IV
1. 10.7-42—6.8-42 . . .	3.4	8.7	11.0	9.8
2. 7.8-42—3.9-42 . . .	4.2	8.7	12.7	10.7
3. 4.9-42—1.10-42 . . .	5.4	5.9	7.3	4.8
4. 2.10-42—29.10-42 . . .	5.4	5.8	10.0	10.4
5. 30.10-42—26.11-42 . . .	6.6	6.1	11.1	10.8
6. 27.11-42—24.12-42 . . .	5.2	5.1	10.7	9.4
7. 25.12-42—21.1-43 . . .	6.8	7.1	10.9	8.7
8. 22.1-43—18.2-43 . . .	10.0	8.3	13.2	12.0
9. 19.2-43—18.3-43 . . .	13.9	12.6	15.4	14.4
10. 19.3-43—15.4-43 . . .	13.5	12.2	15.4	14.2
Total—10.7-42—15.4-43 .	74.4	80.5	117.7	105.2

Group II laid somewhat better than Group I during the first two periods but thereafter the egg production figures for these two groups were

very similar for each period. The egg production figures for Groups III and IV were fairly comparable in all periods, except period 3 when the production in Group IV fell on account of moulting. Group III laid consistently better than Groups I and II. Group IV also laid better than Groups I and II in each period, except period 3. The average egg production figures for the 40 weeks were 74.4, 80.5, 117.7 and 105.2 for Groups I, II, III and IV respectively.

Egg size. Table II gives the average egg size of each Group for each period and the average egg size for the whole experiment.

TABLE II
Average egg size (oz.)

Period	Group			
	I	II	III	IV
1. 10-7-42—6-8-42	1.68	1.72	1.79	1.75
2. 7-8-42—3-9-42	1.81	1.81	1.90	1.87
3. 4-9-42—1-10-42	1.88	1.84	1.91	1.89
4. 2-10-42—29-10-42	1.94	1.95	2.02	1.98
5. 30-10-42—26-11-42	2.00	1.98	2.10	2.05
6. 27-11-42—24-12-42	2.06	2.04	2.14	2.10
7. 25-12-42—21-1-43	2.13	2.12	2.17	2.15
8. 22-1-43—18-2-43	2.15	2.12	2.19	2.17
9. 19-2-43—18-3-43	2.09	2.10	2.19	2.15
10. 19-3-43—15-4-43	2.02	2.05	2.18	2.09
Average—10-7-42—15-4-43	2.00	1.98	2.06	2.04

The average egg size increased steadily in all groups during the first eight periods. Thereafter the egg size in Group I, II and IV decreased considerably until the end of the experiment. Group III maintained its egg size better, and there was only a slight reduction in period 10. The average egg size for Groups I and II was consistently lower than in Groups III and IV. The egg size in Group III was also consistently slightly higher than in Group IV.

Food consumption. Table III gives details of the average daily consumptions of mash, grain, milk and meat offal, the total food consumption per bird per day and the amount of food consumed per pound of egg produced. The average percentage of protein consumed by each group over the whole period is also recorded.

TABLE III

Food consumption and utilization

	Group			
	I	II	III	IV
Av. daily consumption of mash per bird (oz.)	1.99	2.01	2.02	2.03
Av. daily consumption of grain per bird (oz.)	1.96	1.92	1.94	1.9
Av. daily consumption of milk per bird (oz.)	5.10	..
Av. daily consumption of meat offal per bird (oz.)	1.2
Av. daily food consumption per bird (oz.)*	3.95	3.93	4.45	4.17
Percentage protein	10.0	10.0	12.90	13.0
Lb. food consumption per lb. egg produced	7.14	6.90	5.14	5.44

* Milk and meat offal included on a dry matter basis.

Over the whole period the average food consumption per bird in Group III was appreciably greater than that in the other groups. The food consumption in Group IV was also greater than that in Groups I and II. In Groups I and II the average percentage of protein in the food consumed was 10.0, whereas the corresponding figures for Groups III and IV were 12.9 and 13.0. For every pound of food produced the food consumption in pounds in Groups I-IV were 7.14, 6.90, 5.14 and 5.44 respectively.

TABLE IV

Hatchability results.

	Groups			
	I	II	III	IV
No. of eggs set	525	445	715	640
Percentage infertile	12.0	13.3	14.7	9.5
Percentage dead germs to fertile eggs	12.6	8.0	7.5	17.1
Percentage dead-in-shell to fertile eggs	8.6	9.3	7.6	8.4
Percentage hatchability of fertile eggs	78.8	82.7	84.9	74.5

Hatchability. Two White Leghorn cockerels were kept in each of the pens and were moved to each pen in rotation at weekly intervals. Eggs from each group were set every week for a period of eight weeks in a forced-draught electric incubator run at a temperature of 99.5–99.75°F. Details of the hatching results are given in Table IV.

Group IV gave a somewhat higher percentage of fertile eggs but lower hatchability of fertile eggs than the other three groups.

Mortality. The general health of the birds remained fairly good throughout, but many of the birds moulted during periods three to seven. During the course of the experiment, Group III maintained slightly better body weight than the other three groups. The average body weight of the birds in Group I-IV over the whole experiment showed an increase of 9.3 oz., 7.9 oz., 14.8 oz. and 11.0 oz. respectively. Over the period of 40 weeks the mortality percentages in Group I-IV were 18.0, 12.0, 8.0 and 8.0 respectively.

DISCUSSION

The results obtained are broadly in very close agreement with those already reported by Macdonald and Bose [1943] and they prove that a supplement of common salt has little or no beneficial value in laying rations, containing only 10 per cent total protein. This result is somewhat surprising in view of the known value of salt in cereal laying rations, but in this particular instance the protein content of the ration was lower than normal and it is probable that the limiting factor in this case was deficiency of protein and not deficiency of salt. Furthermore, during the whole experiment the feeding of liberal amounts of green food, which is a fairly good source of both sodium and chlorine, tended to make good the deficiency of salt in the cereal ration.

The addition of separated milk and meat offal had a very marked influence on egg production and somewhat improved egg size. Though the milk ration over a period of 40 weeks gave slightly better egg production than the meat offal ration, the difference in egg production was not significant. Both the separated milk and the meat offal rations gave significantly better egg production than the two low protein rations. There was no significant difference in egg size between Groups I and II either for any period or over the whole experiment. Both groups III and IV produced significantly heavier eggs than Groups I and II for the whole period and during most of the ten periods. Though the

average egg size for Group III was somewhat greater than that of Group IV, it is not possible without further experimental work, to draw conclusion in regard to the significance of these differences. It is interesting to note that, with the onset of warmer weather in March and April (period 10), the egg size fell very considerably in Groups I, II and IV, whereas the corresponding drop was very small in Group III. During this period there was a marked reduction in food consumption in all the groups and this probably was responsible for the lowering of the egg size. In the case of Group III, however, the drop in consumption had less effect on the birds probably because they had been regularly consuming greater amounts of food than the other groups. The average consumption per bird in Group III during period 10 was still as high as the average consumption by the other groups prior to the drop in consumption. The birds in Group IV consumed somewhat more food than those in Groups I and II but less than those in Group III. Despite the greater food consumption per bird, Groups III and IV made significantly better utilization of the food consumed than Groups I and II.

In order to test the suitability of the rations for breeders, incubation trials were carried out. Group IV, on the meat offal diet, gave slightly higher fertility than the other three rations. The percentage of dead germs and the percentage hatchability to fertile eggs in Group IV was significantly less than that for Groups II and III. Group I gave somewhat lower hatchability than Groups II and III but better hatchability than Group IV.

SUMMARY

1. Under semi-intensive conditions, a cereal diet (protein content 10 per cent) in conjunction with liberal amounts of green food and calcium is unsatisfactory for laying fowls.
2. Under semi-intensive conditions there is no value in adding common salt to a low protein laying diet composed of cereals plus liberal amounts of green food and calcium.
3. Separated milk is a very valuable protein supplement for laying and breeding birds.
4. Meat offal is a good protein supplement for laying birds but gives lower hatchability than a supplement of separated milk.

REFERENCE

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SELECTED ARTICLES

ARTIFICIAL INSEMINATION IN CATTLE,

A Brief Review of the Technique *

SUMMARY OF CONTENTS

1. Possible applications of the practice in Great Britain.
2. The Artificial Vagina: description of the apparatus.
3. The technique of semen collection.
4. The examination of semen.
5. Technique of insemination.
6. Storage and transport of semen.
7. Note on the viability of spermatozoa in storage.
8. Dilution of semen.
9. Notes on cleaning of apparatus.
10. Apparatus and instrument required.

ADDENDUM.

Technique of making sperm counts

ARTIFICIAL insemination comprises the introduction of spermatozoa into the female reproductive tract by means of instruments, so that direct contact between male and female animals for breeding is unnecessary.

The modern method was first developed in Russia, where it is now practised extensively in horse, cattle and sheep breeding. It has also been used with success in most European countries, and in the U.S.A., the British Colonies and elsewhere.

1. POSSIBLE APPLICATIONS OF THE PRACTICE IN CATTLE BREEDING IN GREAT BRITAIN

In this country artificial insemination has been practised only on a very limited scale. Nevertheless it has several possible applications, which can be summarized under two headings—(a) Livestock Improvement; (b) Disease Control and Increased Breeding Efficiency.

(a) *Livestock Improvement.* It is generally acknowledged that the milk yield of the national herd could be considerably increased by the more widespread use of first-class bulls. The number of such bulls, particularly proven ones, capable of transmitting increased milking characters to their daughters is, however, limited. By the employment of artificial insemination it would be possible to increase considerably the services of such bulls. With proper organisation their services could be made available to a wide circle of dairy farmers who, chiefly for economic reasons, are at present denied such advantages.

(b) *Disease control and increased breeding efficiency.* Certain diseases of the genital organs are transmitted by coitus, e.g., Trichomoniasis, infectious vaginitis, and, possibly, contagious abortion and certain forms of

metritis. This hazard applies within a herd but is greater where communal or inter-herd bulls are operating. Artificial insemination would eliminate the risk of spread of genital diseases by the bull, provided that the animal itself did not harbour any infectious disease which could be transmitted through coitus. In the case of bulls used by co-operative breeding societies, stringent veterinary examination would be necessary to minimize the risk of transmission of genital disease.

Trichomoniasis is a condition which might be cited as an indication for artificial insemination as a measure of disease control. When the disease is well established in a herd, it is certain that the bull will harbour the infection and probably transmit it to clean animals during coitus. In the bull treatment is usually unsuccessful. To resume breeding in the herd without delay it will be necessary to introduce a clean bull, but if he is allowed to serve naturally there is a grave risk that he also will acquire the disease, for it is difficult to ascertain with certainty when a cow which has once been infected is free from the disease.

The adoption of artificial insemination will ensure that the clean bull is protected from infection and thus the continued dissemination of the disease will cease.

Artificial insemination may also be utilized to overcome difficulties in successful breeding caused by some deformities and abnormalities in the genital tract of the cow, which prevent the normal passage of spermatozoa.

The artificial collection of semen provides a means whereby a specimen suitable for microscopical and other forms of examination can be obtained, and thus a check on the fertility of the animal can be kept. This cannot be done with such accuracy where natural service is in use.

2. THE ARTIFICIAL VAGINA (A.V.): DESCRIPTION OF THE APPARATUS

Various methods of collecting semen have been used, but it is now generally accepted that the most satisfactory is the artificial vagina. By adopting it, the whole ejaculate

* Reproduced from the N. V. M. A. Publications No. 11, *Artificial Insemination of Cattle*, published by The National Veterinary Medical Association of Great Britain and Ireland.

free from vaginal secretions and uncontaminated by extraneous matter, can be expeditiously collected.

The Russian model A.V. consists of a hard rubber cylinder, about 24 inches long, into which is fitted a soft rubber tube or lining, slightly thicker than the inner tube of a bicycle tyre. The ends of the soft tube are turned back on the ends of the outer hard tube and fixed in position by binding with half-inch cotton tape. The outer tube has a hole, fitted with a screw stopper, through which water can be poured into the space between the outer and inner tubes, the amount of water being adjusted to give the correct tension on the inner lining. A glass cup with a graduated stem fits into one end of the A.V. and forms a receptacle for the semen. In assembling the apparatus care must be taken to ensure that this cup is firmly secured, otherwise it may be dislodged when the bull thrusts into the vagina. This is especially liable to happen if there is too much water in the A.V.

When assembling the apparatus care must be taken to ensure that the inner lining and all glassware are kept clean and dry. Before handling the apparatus, the operator's hands should also be clean and dry, and without trace of soap or disinfectant.

For filling the A.V., water is heated to about 70° C. (160° F.) in winter and 80° C. (140° F.) in summer, and then poured into the space between the rubber liner and the cylinder. Estimation of the correct degree of tension in the apparatus is a matter of experience, but in most cases this will be attained by laying the A.V. horizontal and pouring water through the filling-hole until the water is on the point of overflowing. Insert the stopper and thoroughly dry all parts of the apparatus with a clean cloth. With a clean glass rod, smear a thin coat of vaseline over the whole surface of the inner lining. Excessive vaseline should not be used as it may run down into the collecting cup and contaminate the semen.

Insert a clean dry clinical thermometer into the open end of the A.V. and leave it in position while the glass cup is being secured. At the time of use, the temperature in the lumen should not be more than 45° C. (112° F.) nor less than 42° C. (108° F.). As there is always some delay between preparation of the apparatus and collection, allowance has to be made for a slight fall in temperature during this interval. The will be greater in winter than in summer. The A.V. may be insulated to prevent heat loss in cold weather by enclosing it in a felt or cloth

jacket. If the temperature in it is allowed to fall below body heat, a few drops of urine may be expelled, and ejaculation may not take place.

3. THE TECHNIQUE OF SEMEN COLLECTION

(a) *Place for collection—Assistance required.* The collection should be made in a yard or field with a level surface and a good foothold. Concrete yards are not suitable owing to the danger that the operator or the animals will slip. It is an advantage if the same yard is used for all services and collections so that the bull becomes accustomed to the surroundings and, when led into the yard, anticipates service. In any case the work should only be done where there is ample space for the operator and his assistants to move well clear should the cow turn. If the operation is carried out in a confined place there is a risk that they will be crushed, or the apparatus broken.

Bulls are handled as for controlled natural matings and should be restrained by a pole attached to the nose-ring. The preputial hairs are usually very dirty and should be washed with warm water otherwise the dirt may get into the A.V. and contaminate the semen. The washing and thorough drying of the preputial hairs should be done before the bull is led out of his box.

The cow used for collection should, if possible, be one in oestrus, and an old cow will usually stand better than a heifer. Often, however, an old quiet cow will stand and allow the bull to mount, even though she is not oestrus; such an animal will prove useful if it is desired to make a collection when there is no cow in heat available. The cow should be haltered and loosely tied up.

Three persons are required, in addition to the operator: one to lead the bull, and one on each side of the cow to prevent her swinging round when the bull mounts.

On some farms service crates are used for ordinary matings but these are usually of a design which is unsuitable for artificial collection; it may, however, be possible to modify existing crates so that they are suitable for this purpose. Edwards and Walton (1938) have described a special service crate for facilitating collection and which may be used with a cow not on heat. These are unnecessary in the ordinary way, but they would prove of great value when a large number of collections have to be made at one centre.

(b) *Collection of semen.* When ready to make the collection the bull is led directly behind the cow. The operator follows the bull

on the right side, holding the A.V. mouth downwards in his right hand. As the bull mounts the A.V. is held behind his foreleg, against the cow's flank, at an angle of 45° with the mouth pointing towards the bull's penis. The penis is directed into the A.V. by the operator holding the sheath with his left hand. It is better not to touch the penis itself with the hand as this may cause the bull to retract and dismount. As soon as the penis comes into contact with the warm lubricated inner surface of the A.V. the bull thrusts vigorously upwards and ejaculates. The A.V. should then be turned with the cup downwards to allow the semen to run into the cup. If the bull has not been used for some time, it is best to make a second collection in a fresh cup and to use this semen for insemination, discarding the first collection. In such a case, the first sample usually contains a large percentage of old immotile or abnormal spermatozoa which have been matured for a considerable time in the epididymes. Also, if the quantity of the ejaculate is small, a second collection should be made immediately after the first.

For ejaculation to occur, it is necessary that the animal shall thrust vigorously into the A.V. Sometimes a bull will introduce his penis into the apparatus several times without ejaculating, and several c.c. of a slightly cloudy and watery secretion will accumulate in the collecting cup. It comprises chiefly secretion from the seminal vesicles and should be discarded. It is probable that conditions in the lumen of the A.V.—either temperature, pressure or lubrication—are incorrect.

After collection the semen should be protected from too sudden cooling in cold weather. It must not be exposed to strong sunlight, which quickly destroys spermatozoa. In the absence of special storage conditions semen is best used for insemination as soon as possible after collection, and it is recommended that it should not be kept longer than three hours.

4. THE EXAMINATION OF SEMEN

Data published by numerous workers experienced in large-scale artificial breeding work in the U.S.S.R. and in the U.S.A. show that great variations exist in the quantity and quality of the semen obtained from different bulls. There is also considerable evidence of variation in the quantity and quality of the semen obtained from the same bull at different times. *A knowledge of such variations and the ability to detect inferior samples is a matter*

of prime importance in the successful conduct of artificial insemination. Consequently the semen of every bull should be carefully examined before it is used for insemination.

The following routine of examination is recommended as being practical under field conditions:*

- (i) Volume of the ejaculate.
- (ii) Colour and density of the semen.
- (iii) Motility of the spermatozoa.
- (iv) Detection of extraneous matter.

The best single index of fertility or otherwise is the motility of the sperm, when examined immediately after collection. It is emphasized, however, that in assessing the potential fertility of a sample of semen all available criteria should be taken into consideration.

Volume of the Ejaculate. This may be conveniently measured in the graduated glass stem of the "Cambridge" pattern collecting cup.

The volume of the ejaculate obtained from different bulls may vary within fairly wide limits. The average, however, may be taken as between 3 c.c. and 5 c.c. when the semen is collected by the artificial vagina method. In general, mature bulls produce a greater volume of semen than young bulls which usually give 1½ to 2½ c.c. Often a bull will ejaculate a small quantity only at the first thrust, but a larger one at the second, taken immediately afterwards.

Colour and density of the semen. These characters can be determined by submitting the sample to a macroscopical examination in good daylight; the examination should not be carried out in strong sunlight.

The normal ejaculate of the bull is an opaque, white or yellowish-white fluid with a milky or creamy appearance. The greater the concentration of spermatozoa, the whiter the fluid. Clear watery semen, sometimes of light yellow colour, contains few spermatozoa. Occasionally a bull of normal fertility produces this latter type of ejaculate and, when such a sample is obtained from any animal, it is essential that a second collection be made: in the fertile bull this second sample should be of normal colour and density.

A decided yellowish or greenish colour may indicate the presence of pus. A dark yellow or brownish discoloration usually indicates the

* In addition to the examination outlined here, the number of spermatozoa per unit volume of semen is of the greatest importance in assessing fertility. This part of the examination is best carried out in the laboratory. For details, see Addendum.

presence of urine which may often be detected also by smell. A pinkish or reddish colour indicates an admixture of fresh blood, while a deep reddish-brown colouration probably indicates the presence of decomposed blood.

Specimens showing abnormal colour or marked deficiency in density should not be used for insemination.

Examination for Motility. A satisfactory examination for motility can be made by placing a drop of semen from a *well-mixed* sample on a slide and placing a cover slip on it. For routine examinations $\frac{2}{3}$ and $\frac{1}{3}$ objective lens are required.

In examinations for motility, the following precautions must be observed: (a) examination should be carried out as soon as possible after collection of semen; (b) care should be taken that it is not exposed to a sudden temperature fall between collection and examination; (c) the sample should be mixed well before taking the drop for examination; and (d) examination should be made in a warm room.

Warming spermatozoa. When examining smears of sperm for motility immediately after collection in cold weather, cooling takes place so rapidly while handling it that often all the spermatozoa appear motionless when examined. Under these conditions it is advisable to hold a lighted match under the slide to warm the spermatozoa sufficiently to make them actively motile, care being taken not to overheat and kill them.

Types of motility. According to Walton [1933], three types of motility may be recognized:—

(i) A progressive motion in which the spermatozoon usually moves in a straight line.

(ii) A rotary motion in which the movement is around the periphery of a circle whose diameter usually does not exceed the length of the individual spermatozoon.

(iii) An oscillatory motion which consists of a series of convulsive motions without change of place.

Walton considers that a spermatozoon must possess progressive motion if it is to meet and fertilize the ovum.

No very satisfactory method of classifying motility has yet been devised, but the following system, introduced by Russian workers, appears to be adequate for practical purposes. It is based on a discrimination between the various types of motility and the proportion of

spermatozoa exhibiting the respective types. Thus: the highest rating, indicated by the figure 5, indicates that all or nearly all exhibit energetic progressive motion; 4, the majority of the sperm have progressive motion; 3, there are about equal proportions of sperm exhibiting progressive motion and oscillatory motion or are immotile; 2, oscillatory motion, with a large number immotile; 1, immotile; and 0, complete absence of spermatozoa.

Adopting the above ratings, it may be taken that bulls giving specimens classed as 5 or 4 are probably of normal fertility; samples classed as 3 indicate bulls of low fertility; while those having ratings of 2, 1 or 0 are sterile.

The same classification may be applied for evaluating the potential fertility of semen after storage.

Detection of Extraneous Matter. Gross contamination of a semen sample with dirt from the region of the prepuce will be recognized on macroscopical examination. Brown muddy discoloration is evidence of this type of contamination.

Examination for cells may be done after suitable staining, but for reliable bacteriological examination, sample are best sent to a laboratory.

5. TECHNIQUE OF INSEMINATION

Semen can be, and often is, used immediately after collection. If it is to be stored or transported to a distance, it requires special treatment (see section dealing with Storage and Transport).

Insemination should be carried out while the cow is in oestrus or within about eight hours after its cessation. If the cow is an uncertain breeder it is recommended that she be inseminated as late as possible in the day in which she is first observed to be in heat, and again the following morning. This will entail keeping semen overnight under suitable storage conditions or collecting afresh.

The cow for insemination should be secured in a stall with, if possible, a good light directly behind her. The lips of the vulva and surrounding area should be thoroughly washed, using a soft brush and warm water. No disinfectant or soap should be used owing to the risk of contaminating and so destroying the sperm. A suitable speculum (Russian or tubular patterns) is inserted into the vagina and held in position with the left hand. For heifers, a smaller type speculum is necessary. The vagina is illuminated with the light from a head lamp or by a

torch held by an assistant. (Several specula are now available in which the light is incorporated in the instrument). Holding the inseminating syringe in the right hand, the nozzle is introduced a small distance into the cervical canal and a quantity of 0.5 to 1 c.c. of semen is deposited directly into the lumen of the cervix. If the sperm is not required for other cows, more than 1 c.c. may be used. The syringe and speculum are withdrawn, and if the cow strains, her back is pinched for a few minutes.

There are various patterns of insemination syringes and catheters, but whichever type is used, it is essential that metal, which is deleterious to spermatozoa, shall not come into contact with the semen. A useful, easily made instrument can be constructed from a 20-inch length of fine-bore glass tube. This should have rounded ends to prevent any possibility of damage to the cervix, etc. The glass tube is attached to a 2 c.c. all-glass syringe by a short length of rubber tubing (Fig. 1) or a rubber bulb

Pressure Tubing



2 c.c. All Glass Syringe
Fig. 1

may be used instead. When using the inseminator shown in Fig. 1 the amount of semen required for use in each cow, 0.5 to 1 c.c., is taken up in the glass nozzle and no sperm comes in contact with the 2 c.c. syringe. This facilitates cleaning and sterilizing of inseminator, as only the glass tubes need to be sterilized by heat and dried before use, and several of these can be carried, a clean one being used for each insemination. This type of inseminator can also be used without a speculum.

The cervix is grasped by one hand per rectum and the glass nozzle of the inseminator introduced into the vagina, and thence through the first or second ring of the cervix, where the semen is deposited.

A 2 c.c. syringe is the most suitable, 5 c.c. and 10 c.c. syringes being too heavy for use. The pressure tubing should be pushed up over the whole diameter of the 2 c.c. syringe, as shown in Fig. 1, as this gives extra rigidity and saves repeated breakages.

An alternative method of insemination has been recently introduced in which the semen is placed in gelatine capsules which have been coated with paraffin wax. The capsules can be inserted into the cervix by means of a capsule "gun" designed for this purpose, or by hand. When the hand is used, liquid paraffin should be used for lubrication. This method is still under investigation and it is not possible to recommend it at present.

6. THE STORAGE AND TRANSPORT OF SEMEN

(a) *For use within three hours.* The precautions to be taken are relatively simple. The semen is transferred to a small, clean, dry test-tube. In warm, summer weather, no special temperature precautions are necessary. In cold, winter weather, the semen must be protected from a sudden fall in temperature; this may be done by placing the test tube in a waistcoat pocket or by wrapping it in cotton wool and placing it under the dashboard of a motor car, etc. At all times it should be protected from bright light and must never be exposed to direct sunlight.

(b) *For use at an interval greater than three hours.* It has been established that cooling is the best method of preserving semen. Spermatozoa normally live only a comparatively short time at body temperature, and room temperature (20° to 21° C.) has likewise been found too high for successful prolonged storage. Most workers are agreed that the longest viability of spermatozoa is secured at a storage temperature of about 5° to 10° C. (40° to 50° F.). In the cooling of semen for storage and its subsequent warming for use, it is essential that the change of temperature shall be gradual, or the sperm will die from temperature shock.

The semen is transferred, using a Pasteur pipette, from the collecting cup to a 5 c.c. Pyrex storage tube. The tube is then filled with liquid paraffin to exclude access of oxygen to the semen. The tube is corked with a rubber or freshly waxed cork stopper.

The tube is wrapped in cotton wool and placed in a tightly stoppered, flat-bottomed container tube, which is immersed for about 30 minutes in water gradually cooled to about 10° C. by adding ice. Finally, the tubes are transferred to a refrigerator or to a thermos food jar or flask containing shipped ice at 5° to 10° C. (Fig. 2). Stored in this way, sperm of initially

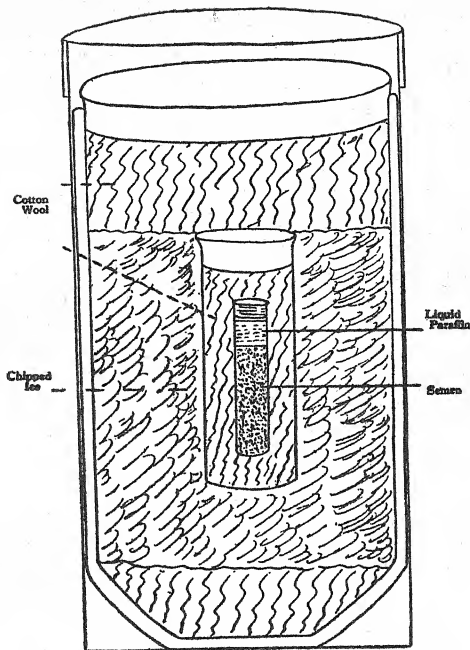


FIG. 2.—Showing method of packing semen for storage and transport.

good quality will retain its motility with little loss of fertilizing capacity for at least 48 hours. For transportation, the thermos flask is placed in a wooden box with hay or straw as packing.

When the stored semen is required for use, the tubes are removed from the refrigerator or thermos and allowed to stand for 30 minutes at room temperature [20° to 21° C. (68° to 70° F.)]. It should be noted that at this stage the tube containing the semen is still insulated by its cotton wool wrapping. This prevents too rapid a rise in the temperature of the semen. A pipette or the inseminator is inserted through the liquid paraffin and the semen withdrawn.

7. NOTE ON THE VIABILITY OF SPERMATOZOA IN STORAGE

There is great variation in the viability of sperm samples in storage from different bulls and also from the same bull at different times. The best indication of high quality semen and its suitability for storage is good motility immediately after collection. (See section dealing with the examination of semen.)

Various workers have reported successful insemination with semen which has been stored for a considerable period. Recently, fertilization has been reported with a sample stored for 198 hours. It is emphasized, however, that the

advisability of using semen stored for long periods is questionable in view of the low ratio pregnancies which results therefrom.

The practical limits of storage for semen are 24 to 48 hours; and, provided the semen shows good motility at the time of insemination, the rate of conception should approximate to that of normal service.

8. DILUTION OF SEMEN

It is emphasized that dilution of semen should be avoided except when it is necessary to inseminate a large number of animals from a single ejaculate, as, for example, in large-scale artificial insemination schemes. The undiluted ejaculate can generally be utilized for the insemination of about three to five cows. The addition of a suitable diluent increases the number of cows which may be inseminated from one ejaculate to about ten.

Special diluting solutions ready for use can be purchased from firms specializing in the supply of apparatus for artificial insemination.

9. NOTES ON THE CLEARING OF APPARATUS.

Artificial Vagina. Before use, new inner timings should be turned inside out and linings should be turned inside out and washing soda has been added.

They should then be rinsed thoroughly in running water to remove all trace of soda and then hung up to dry.

After use, the vaseline which is used as a lubricant must be removed as soon as possible otherwise it tends to 'perish' the rubber. To do this, place A.V. in a bucket containing hot water and washing soda; then with a bottle brush (24 inches long) thoroughly cleanse the inside of the liner. Rinse in water and then swab the liner with a piece of cotton wool or rag soaked in 65 per cent. alcohol; the swab is held in an 18-inch swab-holder.

Syringes collecting cups, etc. Wash all glassware, new and after use, first in cold water, then in hot water and washing soda. Rinse in running water, followed by 65 per cent alcohol. Allow to become thoroughly dry before use by standing on blotting paper or by placing on a warm oven or hot tank.

When not in use all the apparatus should be protected from dust.

10. LIST OF APPARATUS AND INSTRUMENTS

The following is a list of the minimum apparatus required in connection with artificial insemination work. For particulars of prices, the usual firms supplying veterinary instruments should be consulted:

For Collection of semen. One A.V. complete with inner liner and collecting cup; two spare liners; three spare collecting cups.

For Injection of semen. One insemination syringe with spare parts (various patterns). Alternatively, capsules and gun. Speculum; torch or headlamp.

For storage and transport of semen. One dozen Pyrex glass sperm storage tubes (5 c.c.). One dozen glass containers with flat bottom to take the storage tubes. One thermos food jar or flask. One wooden box to hold thermos if this is to be sent by rail etc.

For cleaning the apparatus. One bottle brush (24 inches); 1 swab holder (18 inches). Small bottle brush for cleaning sperm tubes.

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ADDENDUM

Technique of making sperm counts

To estimate the number of spermatozoa in a unit volume of semen the latter must be suitably diluted as that individual spermatozoa may be counted in a cytometer. The Fuchs-Rosenthal and Thoma-Zeiss types are suitable.

A. USING THE FUCHS-ROSENTHAL CYTOMETER

It is necessary to make a preliminary examination under a 2/3 objective to estimate a suitable dilution of the sperm to simplify the counting.

The Fuchs-Rosenthal cytometer is graduated so that the depth of the counting chamber is 0.2 mm. and the area of each small square 1/16 sq. mm.

The sample of semen is well mixed, 0.1 c.c. of semen withdrawn with a fine-bore pipette and added to 9.9 c.c. of normal saline and the resulting suspension well mixed. This gives a dilution of 1 in 100.

For accuracy, it is advisable to have only about five spermatozoa to count in each small square in the cytometer.

This usually necessitates a final dilution of 1 in 800 to 1 in 1,600. The further dilutions are made in multiples of eight, to simplify the final calculation of density per cu. mm.

To convert the 1 in 100 suspensions to 1 in 800, take 1 c.c. of the 1 in 100 and add 7 c.c. of normal saline. At each stage in dilution, the suspensions must be well mixed. The coverslip is placed in position on the cytometer and a drop of the diluted semen is introduced with a pipette under the coverslip and allowed to run in so that it just fills the counting chamber.

The cytometer is then allowed to stand for five minutes to allow the sperm to settle in position. At this dilution, in cold normal saline, they rapidly become immotile.

The count is made under a 1/6 objective and a mechanical stage greatly facilitates the work.

If the dilution is about right (approximately five sperm per small square), an accurate estimate is obtained by counting the number of spermatozoa in three rows of 16 small squares, one in the middle, and one at each end of the cytometer, and taking the average in 16 small squares.

As the volume of each small square is 1/80 cu. mm., the number of spermatozoa per cu. mm. of the semen may be calculated from the following equation, where
= mean number of spermatozoa in 16 small squares,
d = dilution :

$$\text{No. of sperm cu. mm.} = \frac{80x \times d}{16}$$

$$\text{,, ,, c. c.} = 5,000dx$$

B. USING THE THOMA-ZEISS HAEMOCYTOMETER

A dilution of 1 in 200 is suitable. The dilution is made as follows: Thoroughly mix the semen. Then with the Thoma-Zeiss pipette, draw semen, previously well mixed, up to the 0.5 mark. (As the background of the capillary tube is white it will be difficult to observe the semen. This can be overcome by viewing it from a little to one side.) The pipette end is then introduced into normal saline and suction applied until the diluting fluid is drawn up to the 101 mark. No air must enter the pipette during these operations. The mixture is then shaken well in the bulb. A little of the fluid is decanted to ensure that the drop used for the count comes from the pipette bulb. A single drop is placed on the central, ruled area of the counting slide. It is important that the size of the drop shall be such that there are no air bubbles present when the cover slip is applied and also that none of the fluid runs into the channel surrounding the ruled area. With a little practice this is not difficult. The ruled area comprises 16 large squares, each divided into 16 small ones. The volume of each small square, each cover-slip is applied, is 1/4,000th cu. mm.

The number of spermatozoa in 100 small squares is counted (six large squares plus four small ones) using

the 1/6th objective. Of those spermatozoa crossing the lines bounding the squares, only those lying on two sides should be included, so that none is counted twice. In good samples the number in each large square will vary from 15 to 25.

The calculation is as follows: x being the number of spermatozoa in 100 small squares, and the dilution being 1 in 200.

$$\text{No. of spermatozoa per cu. mm.} = \frac{x \times 4,000 \times 200}{100}$$

$$= 8,000x$$

The number of abnormal spermatozoa may be estimated at the same time. These appear in all samples of semen and from animals of all grades of fertility, and it is extremely doubtful whether the percentage of abnormal is of any value in semen evaluation if the total numbers of normal spermatozoa are sufficiently high.

For good fertility, the density should be 700,000 per cu. mm. or higher; frequently, samples from some bulls contain up to 1,500,000 spermatozoa per cu. mm.

NOTE

Certain Breed Societies have drawn up rules governing the artificial insemination of pedigree cows; in view of the temporary nature of some of these rules, any veterinary surgeon wishing to carry out artificial insemination of pedigree cows should obtain from the Secretary of the Breed Society concerned a copy of the current regulations. The names and addresses of those Societies which already have regulations are as follows:—

- Ayrshire Cattle Herd Book Society, 58, Alloway Street, Ayr.
- British Friesian Cattle Society, Aldwych House, Aldwych, London, W.C.2.
- English Jersey Cattle Society, 51, Hillcrest Gardens, Esher, Surrey.
- English Guernsey Cattle Society, Clevedon, Wokingham, Berks.
- Shorthorn Cattle Society, Westfield, Medmenham, Great Marlow, Bucks.

GENETICS OF VIRUSES PATHOGENIC TO ANIMALS*

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THE question: "What is a virus?", is a familiar one, frequently put and still awaiting answer. Are viruses living or dead, animate, inanimate, particulate, biological or biochemical elements, agents, transmitters, enzymes, ferments, catalysts, or what? Without knowing what a virus truly is, considerable knowledge regarding the properties and characteristics of some of the viruses has been gained. It can be said that a virus is a filterable, infective agent, invisible under the microscope; hence the

term ultramicroscopic, filterable viruses. "Filterable" can have only a relative sense and meaning, being dependent upon the nature of the filter and its density, the physical properties of the fluid material, electric charges, and other factors. Moreover, some micro-organisms have filterable phases or forms in their life cycles. The belief that viruses are living agents rests mainly on the fact that they are capable of multiplication and adaptation in the living body and, in vitro, in the presence

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of living cells. Some viruses are infective for susceptible animals in very high dilutions. They are titratable and the minimum infective dose can be determined by tests on suitable laboratory animals according to standardized technique. When an animal or a living chick embryo is infected with 1 to 10 minimal doses, with ensuing fatal results, it is found that the virus has reproduced itself in relatively enormous quantities, a small fragment of tissue yielding billions of infective doses. Perpetuation of the virus and its multiplication at each successive animal passage can be carried forward at will for unnumbered generations or passages. Animals which have recovered from a virus disease or which have survived experimental infection with a virus of modified or attenuated activity are more or less immune to subsequent infection with the same virus. In such cases the serum may contain antiviral bodies capable of neutralizing the activity of the corresponding virus *in vivo* and *in vitro*. A striking feature of many of the viruses is their power of adaptation to foreign hosts and changed environment. Evidence, so briefly indicated in the foregoing, of reproduction, multiplication, adaptation and the development of anti-viral bodies and immuno-biological reactions—corresponding to similar evidence in respect to living bacteria—forms the basis of belief in the living nature and particulate state of viruses from animal sources. But no virus from such sources has yet been obtained in a pure state or dissociated from its co-existence with and dependency upon the complex proteins and molecules of living tissue cells. Until that has come about by cultivation in artificial media in the absence of living matter and more exact criteria are available for determining the size structure and physical properties of viruses, their origin, nature and mode of development lie in obscurity and are open to doubt and question, and to new ideas and conceptions.

Certain virus diseases are associated with the formation of bodies generally referred to as cell inclusions, or virus bodies, occurring in the cell cytoplasm or in the cell nucleus or in both cytoplasm and nucleus. They are more or less pleomorphic, of various kinds and types, differently situated within the cell and peculiar to certain types of cell. Among the best known examples there may be mentioned (1) negri bodies, occurring within the ganglion cells of the brain, particularly in Ammon's horn, of animals affected with rabies; (2) Guarneri bodies, within the epidermal cells in

lesions of vaccinia and sheep pox; and (3) Bollinger bodies, within the epidermal cells of lesions of fowl pox. The nature, significance and relationship of these and other like inclusion bodies to the specific virus with which they are associated is a much discussed question. Their presence or absence may be of much diagnostic importance. Some authorities are inclined to believe that the inclusion bodies and the virus are one and the same, or that the inclusions are bodies which produce the virus. Others view the inclusions as degeneration products of cells attacked by virus. They are not to be observed in all virus diseases but, in fact, only in the minority. They are found mostly in association with neurotropic and dermatropic viruses; and but rarely and doubtfully in typical septicaemic and viscerotropic virus diseases.

Selective affinity for one of the body tissues of its host is a striking characteristic of certain viruses as, for example, the neurotropic viruses of rabies, equine encephalomyelitis and louping-ill of sheep, and the dermatropic viruses of pox diseases. In these the viruses appear to multiply only in the tissues attacked: the brain and spinal cord, the skin and mucous membranes, according to the selective affinities of the respective viruses. On the other hand, in the virus diseases that occur as acute generalized infections, such as hog cholera, South African horse-sickness, infectious equine anaemia, distemper and influenza, the causative viruses multiply in the circulating blood and viscera and are there found in greatest concentration.

There do not appear to be any significant differences between virus and bacterial diseases in the manner of their occurrence and their wide range and variability in respect to contagiousness, infectivity, points of attack, clinical manifestations, selectivity and immunity. As regards allergy and hypersensitivity to virus disease, little, if anything, is known and the question does not appear to have been explored owing, probably, to the difficulties in preparing suitable test reagents.

Ample evidence has been produced to show that a specific virus disease can be caused by strains or types of virus immunologically distinct. This is well recognized and exemplified in foot-and-mouth disease which is caused by at least three virus strains, in South African horse-sickness with its six or more virus strains, and in equine encephalomyelitis with its so-called Eastern and Western types or strains.

Recovery from a specific virus infection leaves more or less immunity against reinfection with the same virus. But where the virus is represented by a plurality of strains, high protection or solid immunity prevails against only one strain. Protection will be slight or, at best, only partial against other strains of the same group. A solid immunity against the homologous strain may therefore prove ineffective against heterologous strains. The coming into existence and development of immunologically distinct strains of a virus may well be conceived as being brought about by changed environment, animal host species and mode of adaptation. Knowledge of the multiplicity of virus strains is growing and it is probable, as investigations continue, that a much greater number than at present recognized, will be uncovered.

One of the greatest handicaps in the study of viruses has been the difficulty in transmitting them and propagating them in laboratory experimental animals. But this is being overcome with increasing knowledge of the susceptibility of the guinea pig, rabbit, white mouse and ferret to certain virus infections and of the behaviour of such viruses in these animals and in the developing chick embryo. Even now there may be more or less difficulty in making the initial transfer from the natural to the experimental host and in obtaining the first "take," which may cause little health disturbance or escape observation. But after a few serial passages the virus adapts itself to the new host and environment with, as a rule, increasing pathogenicity. With some viruses and experimental animals only a few passages or generations are required, and with others many, for the attainment of fixed virulence and type. With certain viruses, such as those of South African horse-sickness, and yellow fever of man, the phenomenon of changing a typical viscerotropic into a fixed neurotropic virus occurs as a result of intercranial inoculations in serial passage, particularly in white mice. Moreover, it would appear that the type of virus which has been developed by adaptation and fixation in the experimental host is maintained and is not reversed when re-introduced to the original host. Further modification is indicated by the observation that a virus, which has attained its highest possible virulence in the passage animal and kills it in a given period of time, is generally less infective and pathogenic for its original host than before its change of type and environment.

In our own distemper studies, virus strains originating from the fox, mink and dog have been maintained for a number of years by continuous serial passage in ferrets. Some minor differences in their infectivity and pathogenicity were apparent at the time they were first obtained, but none could be observed after serial passage in ferrets. And, while the strains are evidently fixed at their highest virulence for ferrets, these animals invariably succumbing to inoculation between the 10th and the 14th day following, usually between the 11th and the 13th day, it is rarely possible to produce clinical distemper with them in either dogs or foxes which were raised under strictly controlled conditions and which, theoretically, should be uniformly and highly susceptible to the disease. Thus, viruses that change in type and reach highest virulence in the passage animals may, at the same time and in the same process, become greatly attenuated for the original host species. Much practical use is being made of this in the preparation of living, attenuated viruses for prophylactic and immunization purposes from the brain tissues of white mice and the embryonic tissues of the developing chick.

Experimenting with the virus of equine encephalomyelitis, we succeeded after several failures in establishing it in guinea pigs by intracranial inoculations of naturally infected horse brain. After a few passages it attained high virulence and appeared fixed as a neurotropic virus for guinea pigs. By similar procedure the guinea pig strain was established and fixed in white mice and, in turn, the mouse strain in the chick embryo. The comparative infectivity and virulence of the three strains so developed in the guinea pig, mouse and chick embryo, respectively, are of significance. Titrating the virus-containing tissues of these animals in dilutions carried to 1: 100,000,000 using 0.1 c.c. of each dilution as the volumetric dose for inoculation, the minimal infective dose of the guinea pig virus is represented as 1: 1000, the mouse virus as 1: 100,000 and the chick virus as 1: 100,000,000. That is to say: the guinea pig virus has 50-100 times greater concentration and infectivity than the horse virus, the mouse virus 100 times that of the guinea pig virus, and the chick virus 1000 times that of the mouse virus. However, such striking differences are probably due to qualitative as well as quantitative factors; and the former, more difficult to put in evidence and to recognize, may be of no less importance in virus investigations, especially in their rela-

tionship to antibody production, serum-virus neutralization and immunity reactions, than the latter. It has already been demonstrated that the chick embryo strain of encephalomyelitis virus can be utilized for immunization purposes far more effectively than the other strains. But whether this is due alone to the great concentration of the virus in the chick embryo or to the concentration together with a change in the character of the virus itself, acquired by sojourn in the chick embryo, is at present an open question.

One more virus disease may be mentioned in concluding this short discussion, namely, infectious equine anaemia, which has points of special interest. It is a highly infective but non-contagious disease, septicæmia-like in character, appearing in either an acute or chronic form and marked by recurring febrile periods which, in the early stages, take place at remarkably regular intervals of approximately 21 days and are suggestive of a cycle not unlike that of certain protozoan infections. The virus is present and apparently multiplies in the circulating blood and has no known special affinities for cellular tissues. The minutest quantity of blood will transmit infection and disease to a healthy horse, and this, with other observations, has led to the strong suspicion that an insect host or vector is responsible for the natural transmission of the disease.

Horses which recover from the disease, and they are many, are apparently immune from further attack but may carry the virus in their blood for the rest of their natural lives and thus become virus reservoirs. As an instance, a horse which had recovered from the naturally contracted disease when about five years of age, lived the remainder of its life at one of our

research stations, passing 20 years of age. At intervals during that whole period its blood proved highly infective for horses and reproduced the typical disease. The fact that apparently normal healthy horses may be and frequently are carriers of infectious-anaemia virus is not to be overlooked when horses are used for the production of anti-sera intended for either human or animal use.

Inability to infect any species of laboratory animal or to propagate the virus of infectious equine anaemia under laboratory conditions has hindered the investigations we have carried on for 25 years. But with improved methods of study recently introduced and experience gained with regard to susceptibility of mouse, ferret and chick embryo to virus infections, there are increasing possibilities of propagating this virus in the laboratory and of studying it to better effect.

Many other diseases in which the etiological agent is filterable and ultramicroscopic occur in North America. Many more, unknown on the North American Continent, are frequent in either Europe, Asia or Africa. These virus diseases are just as important, economically, as the diseases of known bacterial origin, with which they have much in common. The view that viruses are animate, particulate elements and agencies appears to be well taken and doubtless will be held until satisfactory evidence to the contrary has been produced. Their orderly classification, powers of multiplication and adaptation, as well as other inherent properties and affinities will for a considerable time to come hold the attention of virologists and, perhaps the interest and scrutiny of geneticists.

THE STANDARDIZATION AND INTERPRETATION OF THE AGGLUTINATION TEST FOR *BRUCELLA* INFECTION IN CATTLE*

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The Secretary of Section L has asked me to lead the discussion on the subject of "What constitutes a reaction to the agglutination test for contagious abortion?" At first glance it might appear that a certain degree of agglutination and sedimentation of the bacteria constitutes a reaction, but in the diagnosis of infection the matter is by no means so simple.

It appeared to me, therefore, that a general discussion of the development of the test and the various factors involved would be of some value.

Topley [1933] records that agglutination of bacteria was first described by Gruber and Durham in 1896. Grunbaum [1896], Widal [1896] and Widal and Sicard [1897] recognized the value of the test for the diagnosis of typhoid

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fever, but its use was soon extended to other diseases, though often with indifferent success [Arkwright, 1931]. In addition to the testing of sera for the presence of agglutinins against bacteria suspected of having invaded the host, agglutination was utilized in the identification of unknown bacteria.

Specific agglutination is now regarded as occurring in two stages. In the first place the bacteria are acted upon by the agglutinin present in the serum of the infected or immune animals. This action confers on the bacteria the property of clumping in the presence of neutral salts in low concentration. In the second place the bacteria are agglutinated into clumps in the presence of electrolytes. The clumps then fall to the bottom of the tube by gravity.

Wright and Smith [1897] used the agglutination test for the differentiation of Malta and typhoid fever. Birt and Lamb [1899] described the agglutination test for Malta fever, basing their work on that done previously by Wright. These authors recognized the necessity for a standard method of preparing antigen, a knowledge of the agglutination titres of sera of normal and infected individuals and a knowledge of the period that elapsed between infection and the presence of agglutinins in the serum.

Since this work was published the agglutination test has come into general use in the diagnosis of infection of cattle with *Brucella abortus*.

McFadyean and Stockman [1912] concluded as a result of their tests that "one will be justified in regarding complete agglutination with a serum dilution of 1 in 50 or 1 in 100 as strong evidence of infection." They drew attention to the fact that all animals whose sera give a positive reaction do not necessarily abort and that certain infected animals which do abort may not give a positive reaction at or shortly after the event although later the serum will react positively. The standardization of the bacterial suspension used in these tests was "when viewed in one of the small tubes employed for the tests, it was faintly hazy in appearance." The tubes were incubated at 37°C. to 38°C. for 24 hours before reading.

Seddon [1915] reported upon his investigations into use of the agglutination test. He emphasized the necessity for a standardized bacterial emulsion for use as antigen, using a suspension of barium sulphate† as a standard

†Three ml. of 1 per cent solution of barium chloride in distilled water was added to 97 ml. of 1 per cent solution of sulphuric acid in water.

of capacity. In a later report Seddon [1919] drew attention to the fact that, whilst agglutination due to non-specific agglutinins would take place if sufficient serum was added to the bacterial suspension, the concentration of these agglutinins is never very high. With regard to the agglutinins developed as a result of invasion of the body by *Br. abortus*, he referred to the definite relationship between the quantity of specific agglutinins and of the bacterial suspension which go together to produce the agglutination reaction, pointing out the necessity for using a definite unit of the suspension in the test.

As a result of his investigations he concluded that agglutination with 0.1 ml. of serum, using the unit of antigen previously described, was evidence of infection (*N.B.*: 0.5 ml. of suspension was used and the total volume of fluid in the tubes was 2 ml.). Seddon compared the agglutination responses with the clinical history of the cattle. Like McFadyean and Stockman, he noted that some infected animals failed to give a positive reaction until after abortion and that a small percentage of cattle, infected apparently in the uterus only, may have a decreasing agglutination titre and eventually give a negative reaction.

The agglutination tests carried out at the Glenfield Veterinary Research Station are based upon Seddon's work, agglutination with 0.01 ml. of serum being regarded as a positive reaction. For purpose of convenience, however, we standardize the antigen by comparison with tube 2 of Burroughs Wellcome standard opacity tubes. This gives a suspension slightly less dense than Seddon's Barium Standard.

The wide use of the agglutination test for the establishment of abortion-free herds has led to many thousands of tests being carried out in laboratories in almost all civilized countries. From the accounts of tests as applied in various centres, there is considerable variation in the technique adopted, and unless the exact details of the test are published it is difficult to make comparisons between the results obtained by different workers. Hence it is now generally admitted that there is necessity for the determination of a standard for the technique and interpretation of the test. In this connection Stableforth [1936] has published some interesting and pertinent data. He carried out tests on the sera of 50 cows, with the bacterial suspensions of different laboratories and using the technique and methods of reading adopted in

these laboratories. The results were far from uniform.

19 cows reacted negative or doubtful.

24 cows reacted negative or doubtful or positive.

2 cows reacted doubtful or positive.

1 cow reacted negative or positive.

4 cows only gave a positive reaction to all the methods used.

Thus 46 of the 50 cattle would have been judged positive, negative or doubtful according to the technique adopted.

Similarly in America sera have been examined simultaneously in a number of laboratories with reactions that varied to a considerable degree. When one considers the large number of institutions throughout the world in which the test for Bang's disease is carried out, the almost innumerable variations in technique and interpretation, and the fact that, in spite of these variations, herds are being rid of the infection, we can only marvel at the results being attained. However, it is undoubtedly true that the less exact methods lead to economic loss in causing the disposal of non-infected animals and in lengthening the period of eradication by the failure to select some infected cattle.

It is obvious that in deciding the significance of the results of the test we are not concerned merely with the highest or lowest dilution which gives agglutination; we have to take into consideration:

(a) The suspension of bacilli.

(b) The blood serum.

(c) The diluting fluid.

(d) The treatment of the combination of serum and antigen.

Frei [1937] discussed these matters very fully, and his opinions are freely quoted in the following summary.

Serum. Coagulation can exercise an action on the colloids of the serum and notably on the agglutinins. Coagulation at low temperatures or shaking during coagulation must be avoided, otherwise the agglutination titre will be lowered. Tests should be put up as soon as possible after collection of the serum, since the titre may fall if the serum is held for lengthy periods, although delay of three or four days is not of much significance. How far the holding of uncontaminated sera is of importance is difficult to state, but the sera used as known positive controls at Glenfield have been for some months carbolized without any appreciable loss of titre in the diagnostic

range. These sera are, however, strongly positive, and it can be understood that a slight loss of agglutinin may be of significance with sera on the border line; of course contamination of the sera, which frequently occurs in field collection and at high temperatures, exercises an adverse influence and renders some sera untestable. Similarly haemolysis and consequent staining of the serum is undesirable, and according to Frei may lessen the titre. In our experience haemolysis frequently leads to a deposit in the control tube not containing antigen and thus renders the interpretation of the test very difficult. Frei, quoting the report of the U. S. A. Commission on Bang's Disease, states that, whilst variations of pH within fairly wide limits (4.7 to 8.9) do not have any noticeable effect on the titre, unclean bottles, especially those which have held disinfectants such as cresol or soaps, tend to lower the titre.

Bacterial suspension. It is generally known that the agglutinability of the antigen is influenced by the method of culture and type of culture media employed. Stableforth showed that when using the same bacterial strain there was a difference in the agglutinability of suspensions prepared in the different media, prepared in similar media but using organs from different species of animals, and even in different flasks using the same medium. Hence it is well-nigh impossible to guarantee that supplies of media prepared under similar conditions will be identical. Such difficulties could be overcome if each batch of antigen could be titrated against a standard serum; the recent work in the preparation of standard dry serum offers hope in this direction.

Whilst many laboratories prepare their antigens from cultures when they have attained their apparent maximum growth, e.g., after two or three days. Frei prefers to use 24-hour cultures, since, in his opinion, at this age of culture there is maximum agglutinability. Culture is sometimes raked off the medium with a glass rod or a platinum needle. In the U. S. A., however, the usual practice is to flood the flask or tube with carbol saline, and allow the fluid to remain in contact with the culture two or three hours before washing off. In any case the suspension must be filtered to remove any particles of agar, which, if left in the antigen, may increase the degree of agglutination.

The use of living bacteria in the suspension is favoured by some, but the consensus of

opinion is against this practice on account of danger to the laboratory staff, and because the bacteria may be killed without any appreciable effect on agglutinability. In this connection Henry and Traum have stated that tricoresol 0.2 per cent and carbolic 0.5 per cent have scarcely any effect on the results of the test, but that formalin is not desirable.

As previously mentioned the density of the suspension is very important. Various methods have been adopted in the fixation of a standard of density. Opacity standards, enumeration of bacilli, centrifugalization, nephelometers and opacity meters have all been used. Probably the best method is by centrifugalization, using Fitch's modification of Hopkins' vaccine tube. Whilst some writers have stated that within certain limits the density of the suspension is without influence on the results of the test, others, notably Fitch, Donham, Bishop and Boyd, are of opinion that the maximum titre of agglutination of sera containing a moderate quantity of agglutinin changes with the density of the antigen. Stableforth has shown that with an increase in the density of the antigen there was a diminution in the titre of the serum.

The agglutinability of strains of *Brucella* from different sources varies to a great degree. Traum and Henry tested the agglutinability of 22 strains of *Br. abortus* and found considerable variation. Frei states that many strains react with sera which give negative results with other strains. On this account most workers use a suspension made of a number of strains.

In putting up the test it is usual to use a series of serum dilutions, although in the routine testing of large numbers of sera the series is a small one. Some workers make their serial dilutions in normal saline or carbolsaline before adding the sera to the antigen. Others, notably in America, add undiluted serum direct to the antigen. Stableforth states that with the latter method higher titres are obtained. The actual volume of fluid in the tubes is of some importance, as agglutination is more rapid in the smaller volumes, although the final result is probably the same.

The tubes are usually incubated at from 37° C. to 40° C., although agglutination will take place at lower temperatures. Frei agrees with the finding of the Commission on Contagious Abortion of U. S. A. (1932-33) that the test should remain in the incubator 42 hours at least.

READING THE TEST

On reading the test it is usual to refer to different degrees of agglutination, indicating the reaction by the sign +.

	Continental	Fitch U.S.A.
Complete sedimentation, supernatant clear	++++	+
Almost complete sedimentation, supernatant almost clear	+++	1
Much sedimentation, supernatant hazy	++	
Light sedimentation, supernatant cloudy	+	
No sedimentation	::

INTERPRETATION OF THE TEST

With regard to *Brucella* infection of cattle, we must bear in mind that:—

(a) Agglutinins may appear in the blood in from one to three months after infection, so that an animal may be recently infected and give a negative reaction.

(b) Agglutinins may persist in the blood for a long period, even if the infection be lost, so that all animals giving a positive reaction do not necessarily harbour the living organism.

(c) The intensity of the reaction does not necessarily indicate the virulence or degree of the infection.

(d) There is a relationship between continuous high titre and under infection.

(e) The greatest percentage of abortions occur in animals with a high titre (Huddleston).

Willems [1937] suggests that an agglutination titre should be determined, and he recommends the calculation of a constant of agglutination. He takes as an index of agglutinability of the suspension that dilution of a standard positive serum which gives 50 per cent of agglutination.

Calculating in this way he found that 50 per cent agglutination was given in a serum dilution of 1 in 700 with the suspension used in his laboratory, and with 1 in 400 with the suspension used by Stableforth.

The indices of agglutinability therefore were 700 for the Belgian suspension and 400 for the English suspension.

This index is a measure of the degree of sensitivity of the suspension.

Utilizing this suspension with an index of agglutinability of 700, Willems regards as a positive reaction a 50 per cent agglutination with a serum dilution of 1/50.

The constant of agglutination is calculated as 50/700 or 0.07142.

Willems found that the index of agglutinability of the English suspension was 400 and the titre indicating infection will be obtained by multiplying the index of agglutinability by the constant of agglutination $400 \times 0.07142 = 28.56$, or roughly 1/30.

Stableforth regarded a positive reaction as that of 25 per cent agglutination with a serum dilution of 1/40.

With the standard serum and the English suspension 25 per cent of agglutination was obtained with a serum dilution of 1/500.

The titre of infection, therefore, is $\frac{500 \times 28.56}{400} = 1/35.7$, which is very close to Stableforth's standard of 1/40.

Willems points out that this shows that the infection titre recognized by him is close to that adopted by Stableforth, but he stated that it is not possible to forecast the titre of a serum with mathematical precision.

He tested the method on 11 sera:—

In 3 cases the titre was accurately forecast.

In 6 cases the titre was 10 per cent in error.

In 5 cases the titre was less than 10 per cent in error.

In 1 case the titre was less than 20 per cent in error.

In 1 case the titre was less than 50 per cent in error.

How far such a method would be found of value it is difficult to say, but Willems' own test, although limited, shows a considerable margin of error in his prediction.

It is obvious, however, that it is not possible to answer the question "What constitutes a reaction to the agglutination test" until we know: (1) the exact technique of the test; (2) the agglutinability of the suspension used.

So far each laboratory appears to have worked out its own system of technique and interpretation on an empirical basis, although in some cases the titre indicating infection has been arrived at by reference to the clinical history of infected animals and subsequent bacteriological examinations.

Having adopted a standard method of antigen preparation and technique of test and having determined the titre which is indicative of infection, we are still faced with the problem of those animals whose sera have a titre somewhat below the accepted diagnostic titre and yet above that which might be ascribed to non-specific agglutination. Donham and Fitch have investigated this matter. They point out that the agglutination titre or titres which are selected as a basis to differentiate

between specific and non-specific agglutinins are arbitrary separations in a continuous scale of agglutination reactions extending from low to high dilutions of serum. They have been made after extensive studies and experience, but they are not infallible. These authors tested 303 sera from cattle in herds that did not contain any animals with positive agglutination titres, with dilutions of 1 in 10 to 1 in 25. Thirty-seven per cent of these showed agglutination in a dilution of 1/25. The significance of their findings was that we cannot consider all agglutination reactions in low dilutions as evidence of Bang's disease, although it may be an indication of a rising titre in a recently infected animal. Donham and Fitch do not consider this latter possibility of much importance as it detects only a minor percentage of animals that should be regarded as suspicious. In America the titre indicating infection is regarded as 1-100. Taking Donham and Fitch's work, it appears that somewhere between the 1/25 dilution and the 1/100 dilution, agglutination which may or may not be the result of *Br. abortus* infection will occur. Since the fixation of the titre of infection is arbitrary, we have to recognize that some reactions must be regarded as suspicious because we are not justified in diagnosing them as positive or negative. Subsequent tests of sera of the animals from which such sera have been collected will indicate if the titre is rising and thus permit a definite diagnosis. Kitzelman [1936] reviewed the after-history of 1,000 cows testing suspicious (1/50) to the Bang agglutination test. After five months 60.1 per cent had become negative, 23.1 per cent had become positive, and 16.8 per cent remained unchanged. These cattle were in herds in which the percentage of infection at the initial test ranged from 10 to 17.9 per cent. Thus 82.2 per cent of the suspicious reactors gave a definite negative or positive reading within five months of the doubtful test. There are, however, certain animals of which the serum titre persists at the one level of suspicion. In our experience such animals are not infected, although a careful bacteriological examination of the animal after slaughter has only been possible in one or two cases.

In determining the possible danger of these animals most authorities are of the opinion that the interpretation of the agglutination results can only be made satisfactorily in the light of the disease status of the herd, the breeding

history and the stage of gestation. Thus Donham and Fitch would prefer to accept as non-infected a cow showing a low agglutination titre from a herd with no positive reactors than a non-reactor from a diseased herd.

All that has been said so far refers to the microscopic tube agglutination test as carried out in the laboratory.

Attention is drawn to the rapid tests which have been evolved in other countries, particularly America. The rapid plate agglutination test is being used in at least one State in the eradication of *Brucella* infection of cattle, and in other States it is playing some part in the control schemes. It is claimed that with this test quantities of serum approximately equal to those used in the tube test are employed and that the results are very reliable. Of course, if this test were adopted a standard antigen should be available to all veterinarians using it, and a standard of technique and interpretation would be required.

CONCLUSIONS

There is no generally accepted standard for the performance and interpretation of the agglutination test for the diagnosis of *Brucella* infection of cattle.

Some attempts have been made to evolve a method of standardization of this test.

A standard of preparation of the bacterial suspension should be adopted in Australia, and the agglutinability of each batch be tested against a standard positive serum.

The standard positive serum can be maintained at approximately its original titre for some years in a dry condition.

It would be an advantage if the standard suspension to be used throughout Australia could be prepared in one central laboratory.

A standard technique should be adopted throughout Australia so that the tests carried out in different laboratories will be comparable.

Consideration should be given to the adoption of the rapid methods of agglutination testing since these would be of value to the field officer in the initial determination of the approximate degree of infection in a herd. It may be possible to adopt this method as the standard test as has been done in parts of America.

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ABSTRACTS

Report on experimental commercial preparation of butter fat at Longwarry : August to October 1941. G. LOFTUS-HILLS (1941) *Report of the Coun. Sci. Industr. Res. Australia* 22

THIS report deals with a description of the experimental plant set up at Longwarry, for the larger scale manufacture of butter fat from surplus second grade butter of Australia, with the use of centrifugal separation and vacuum evaporation for the removal of water. The butter fat was sent to Great Britain and to the Navy. The butter was melted in half ton lots by direct exposure to steam (25 minutes) and then allowed to pass through two Alfa-Laval No. 85 whey separators, set to give about 55 per cent cream from whey. The flow of butter fat from the second separator was regulated to give a fairly cloudy, but not completely opaque, butter fat. The butter fat from the second separator was afterward

dried by heating under reduced pressure in a modified Vaeorator unit and then passed through the Internal Tubular type of cooler. The butter fat was settled in a stainless steel tank and finally filled as completely as possible, and packed in 4 gallon tins of which the 2½ screw top type with push in bungs was found to be most satisfactory. At a vacuum of 28.5 to 29.0 inches of mercury, the evaporator removed 2.0 to 2.5 per cent of water from the fat at maximum throughput. It is possible to dry to 0.1 per cent of moisture content, the plant working at 1½ tons of butter per hour. An approximate estimate of the general order of the working costs reveals that the cost of the product per lb at the place of manufacture is equal to the cost of butter per lb. delivered to place of manufacture 100/80-2±1.4d. The rate of separation was markedly increased by adjusting the pH of butter serum, after melting to 10.0 with caustic soda. The use of unsalted butter did not have

any marked effect on the speed of separation, but the amount of curd deposited in separator bowls was much reduced. Increase in the amount of serum solids retained by the final fat between 0.1 to 0.2 per cent was generally associated with improved flavour of the fat. Decantation of a part of the serum did not reduce the curd deposition in the separator. Dilution by condense water in direct steam melting was found to be about 17 per cent. Coefficients of heat transfer from fat to metal were given. The bacterial count of the fat per ml. was below 10. The contamination of copper and iron and the need to avoid them are dealt with. The analytical methods for the determination of water (Karl Fisher method) copper and iron (McDowell's modification of Moir's method) are described. Cooling to about 70°F. in tins was necessary to get a desirable texture for the butter fat. About 14 tons 4 cwt. of butter was handled. [C. P. A.]

Yield, chemical composition, and feeding value for milk production of alfalfa hay cut at three stages of maturity. *Tech. Bull. U. S. Dep. Agric.* No. 739, 1940, 1-51

The effect of cutting alfalfa at different stages of maturity on its yield, chemical composition, colour, leanness and relative nutritive value as determined by feeding experiments and digestion trials on sheep and Holstein cows is given. Cutting alfalfa at 3 stages of maturity, namely (a) initial bloom stage when not more than 10 per cent of the plants are in bloom, (b) at half bloom stage when about 50 per cent of the plants are in bloom and (c) at full bloom stage when 90 to 100 per cent of the plants are in bloom appears to have significant effect on the quality. Alfalfa cut at the initial or half bloom stage was markedly superior in the yield of hay; (8938 lb. and 8888 lb. as against 6940 lb. per acre) and yield of digestible nutrients per acre (4660 lb. and 4413 lb. as against 3260 lb. per acre per year) to the alfalfa cut at the full bloom stage. The line of demarcation between the first and second stage is not very significant.

With sheep as experimental animals, the average digestion coefficients obtained for crude protein were 77.7 per cent for the initial bloom hay, 77.1 for the half-bloom hay and 75.4 for the full-bloom hay. The corresponding digestible coefficients for crude fibre were 47.7, 41.4 and 38.3 per cent respectively. Experiments on 3 Holstein cows fed exclusively on initial bloom of alfalfa hay showed an average production of 11,099 lb. of milk and 404 lb. of butter fat (calculated on mature age basis). The cows on half bloom hay produced on an average 9763 lb. of milk and 345 lb. of butter fat (mature age basis). The 4 cows on full-bloom hay produced an average of 8981 lb. of milk and 331 lb. of butter fat on mature age basis. The rate of consumption of initial bloom-hay per pound of milk and butter fat produced was less than that of half and full bloom hay. The calculated amount of milk produced per acre was 6330 lb. for initial bloom plot, 5234 lb. for the half bloom plot and 3970 lb. for the full bloom plot after the actual quantity of milk produced was corrected to 4 per cent butter fat. [M. C. R.]

The feeding of dairy cows for intensive milk production in practice. E. MEIGS (1939). *U.S. Dep. Agric. Yearbook Agric.*, 566-91

The importance of roughage and concentrates in the feeding of dairy cows, the proper way of feeding and the determination of the amount of food required are given.

The vital importance of good roughage, concentrates and pasture for maintaining the high producing dairy cow is stressed. Half and half of legume hay (alfalfa) and grass hay (timothy) are recommended for high milk yielders. A good concentrate mixture can be made up of 4 parts of corn meal, 3 parts of wheat bran and parts of one or more of the oil cake meals plus 1 per cent of salt. Feeding cows according to the Savage Standard which is very liberal standard, appears to be the best. To maintain uniform body weight, heavy milking cows, in the early part of the lactation, should be fed *ad libitum* hay and as much concentrates in addition as they would clear up in half an hour twice a day. For successful reproduction, cows need about 10 p. m. carotene in the dry matter of the ration. Tender grasses or legumes high quality alfalfa meal and cod liver oil are good vitamin A concentrates. Cod liver oil in large quantities appears to be harmful to cows. It reduces the fat content of the milk. Mature cows can be fed two ounces of cod liver oil per day. Heavy milking cows require about 0.25 per cent each of calcium and phosphorus in the dry matter of the ration. Iodine deficiency is not frequent in dairy cows. The author discourages the practice of administering mineral preparations and vitamins to make up for poor rations. [M. C. R.]

Studies on the nutritive requirements of Bacteria. WOLLEY (1941). *J. Bact.* 42, 155

The author describes some of his interesting researches regarding growth-factor and amino-acid requirements of certain bacteria.

He found that the addition of riboflavin, pantothenic acid and a suitable reducing agent to a tryptone and liver extract medium, inactivated with alkali, could restore the original effectiveness of the medium for the growth of haemolytic streptococci. He then attempted to grow a member of group D of haemolytic streptococci on a synthetic basal medium containing hydrolysate of purified casein, glucose, inorganic salts, riboflavin, pantothenic acid and reduced iron and observed that the addition of vitamin to this medium was essential for the growth. Then the only unknown constituents of this basal medium were the amino-acids of casein hydrolysate. He also discovered that substitution of a mixture of 19 pure amino-acids in place of the casein hydrolysate gave good growth. Glutamic acid, tryptophane, isoleucine, lysine, arginine, cystine and tyrosine were, on the other hand, found, to be essential for growth; mixture of these 7 amino-acids produced a good growth of bacteria. But some closely related strains failed to grow on this simplified mixture of amino-acids and required a larger variety of amino-acids and nicotinic acid.

Only the members of groups B and D of haemolytic streptococci would grow on this purified medium. For the growth of a member of group A, further addition of an unknown water soluble factor of liver extract was necessary.

The attention of the author was then drawn to the fat soluble growth-factors and he found that many such compounds possessing vitamin K activity were active for the growth of *John's* bacillus.

He finally points out that the same compounds are concerned in the growth of all living cells, and the use of bacteria, rather than rats and other animals, for the assay of vitamins would materially reduce the cost of the labour involved in the analysis of these substances. He also mentions how the studies on microbial nutrition helped in the discovery of the chemical structure of pantothenic acid. [N.B.D.]

The inheritance of equine coat color. The basic colors and patterns. G. W. SALISBURY (1941). *J. Heredity* 32, 235

THREE sources were used in obtaining information about the inheritance of the more common colours, some specific colours and face and leg markings of the horse. These sources were the American Shetland Stud Book, the Studbook for Finnish Horses and the records of the Jockey Club. Varying amounts of data on body colour, colour of mane and tail, white markings and other patterns are presented.

Basic or foundation colours are listed as bay; brown; brown-black or seal brown; black; and liver chestnut; chestnut and sorrel. Colour may vary with age and exposure to sunlight, making it difficult to properly classify horses on the basis of colour.

Undoubtedly (1) brown-black ponies were classified frequently as black instead of brown and (2) colts were improperly classified before the shedding of the foal hair whose colour may differ from the basic body colour. Despite these possible errors, brown is an important colour in the Shetland breed. Furthermore, different shades of chestnut were not distinguished by Shetland breeders. However, a study of 3,235 Shetland matings indicated that the mode of inheritance of the basic body colours is the same in this as in the larger breeds of horses.

Wentworth reported that the light mane and tail of the Belgian horse were due to a monofactorial recessive to the normally pigmented mane and tail. The present study suggests, however, that it is more complicated, involving at least two recessive pairs of homozygous genes, with additional genes which modify the shade of the coat and also tend to influence the amount of pigment in the mane and tail. The white mane and tail seem to have the same inheritance in the chestnut as in the sorrel.

Genes which are epistatic to the basic colours are 'those responsible for the grey colour, roaning, spotting, dominant white colour, and dilution of the basic colours. Observations on 106 matings indicate that grey is produced by a dominant gene which is epistatic to the basic colours. The roaning gene acts similarly. There are two types of white spotting, each with a different inheritance. Extensive spotting is due to a dominant white spotting gene, whereas limited spotting seems to be due to more than one pair of genes which may involve both dominance and recessiveness. Slight breed differences were noted which may be due to the 'unconscious selection practised in the development of these breeds.' A single dominant gene, epistatic to all colours, is indicated in certain white horses although this condition is confused with both old homozygous gray and the results of the dilution gene on chestnut or sorrel. The dilution gene is not discussed in this paper. [J.N.W.]

The inheritance of equine coat color. II. The dilutes, with special reference to the Palomino. G. W. SALISBURY and J. W. BRITTON (1941) *J. Heredity* 32, 255

THE Palomino is characterized by a cream-coloured body, varying from a dark gold to a light, washed out yellow, with a white mane and tail. The eyes show a bluish-pink appearance, the skin is pink.

Certain previous workers called this colour cream, as distinguished from dun which referred to horses with a light body colour, black mane and tail, a dorsal stripe and often with 'Zebra-striped' legs. Those kinds of a more yellow colour are commonly called buckskin.

Wredit is reported to have felt that cream was an unfixable heterozygous condition, this theory is substantiated by breeding experiments. Heizer considered that dun, mouse, and cream are produced by a dominant dilution gene superimposed over certain basic body colours'. Tuff accepted this postulate but considered that a pair of genes, responsible for the quantity of pigmentation, was involved. In the homozygous recessive condition this pair produced albino; he called it the albino factor. In the heterozygous condition the pair reduced chestnut to cream, bay or brown to buckskin, and dun to light dun, but had no influence on black or mouse'. His dilution gene, therefore, was dominant.

Gremmel, on the other hand, concluded that the albino was a homozygous recessive for genes at three major loci of which one dominant at the first locus produced a Palomino, while two dominant genes at this locus gave a red ysabella or sorrel. He denied any relationship of the Palomino to dun.

The authors of the present study were confronted, therefore, with two postulates: one explaining Palomino by the basic chestnut or sorrel plus a dominant dilution gene, which also dilutes bay or brown to dun, and black to mouse; the other being chestnut or sorrel possessing a recessive member of a pair of genes responsible for basic pigment.

A study of the American Shetland Stud Book and visits to Palomino breeding establishments provided material for the present work. The Shetland pony data indicated a dominant gene was responsible for cream, dun and mouse colours. Matings involving two animals with the dominant dilution gene gave mostly dilutes, where one parent was a dilute and one solid-coloured, the ratio of dilutes to non-dilutes was very close to the expected 1:1. No differences were found between creams and Palomino so far as colour, type or distribution of colour was concerned. A relationship between these two colours, therefore, seems obvious. Visits to some Palomino breeding establishments as well as reports from others unvisited indicate that 40 to 50 per cent of the progeny of a Palomino stallion and chestnut or sorrel mares were Palominos, the rest were chestnut or sorrel. Breeding work of troop D, New York State Police at Oneida, New York, gave similar results.

It is concluded, therefore, that the Palomino is produced by the presence of a diluting gene which does not mask the effects of genes which cause intensity variations. A basic chestnut with a white mane and tail plus the dilution gene gives the most desirable coloured Palomino. When the gene for black is absent, the homozygous intensity reduction condition gives an individual with practically no pigment in the hair. When these are crossed on other colours the progeny all show a reduction of the hair pigment. It is not felt that where the gene for black is present, the homozygous dominant dilution condition produces an albino; the writers believe that such a case results in a washed-out, light dun. It appears that only one pigment reducing gene was involved in the horses studied. [J.N.W.]

The chromosome complex of domestic sheep (*Ovis aries*) R. O. BERRY, (1941). *J. Heredity* 32, 261

SEVERAL workers have reported on the number of chromosomes in the domestic and wild sheep. Their observations were made largely on testicular material prepared by standard methods, or on the amnion. Most of them reported a diploid number of 54 or 60. With one exception, testicular material was used in cases where

60 are reported and amnion preparations in cases of 54. A study of the chromosome complex of germ cells, therefore, was undertaken in the hope of establishing the exact number of chromosomes present in sheep. Modern techniques were employed.

Chromosome counts were made on more than 65 spermatogonial cells, taken from lambs which had just attained sexual maturity. Many of the chromosomes in these cells showed very little over-lying or touching so that they were easily distinguished one from the other. The diploid counts were consistently 54. Metaphase plates of the primary spermatocytes contained 27 chromosomes. Morphologically the chromosomes of the sheep consist of a sphere; rods which may be long, short or bent; and large V's. These latter vary somewhat in size and symmetry, but were distinctly observed to be V-shaped single chromosomes and not two rods so arranged as to give the appearance of a V by the over-lying of one end of each. Since there were three pairs of these present involving six individual chromosomes, the authors suspect that other workers may have considered them each made up of two rods over-lying at one end thus explaining counts of 60. The remaining 48 chromosomes consist of two pairs of slightly bent, medium sized rods, 43 straight rods varying from 4 to 1.5 microns long, and one sphere. Observations on the anaphase showed this one sphere paired with a medium sized rod.

Considerable difference of opinion exists in reference to which chromosomes constitute the sex or X-Y chromosomes. The authors of the present study believe the heteromorphic pair, viz. the medium sized rod and the small sphere, are the X-Y chromosomes. They do not think that the fact that different workers have studied three breed groups, two wild species, and hybrids of these wild species with domestic sheep explains the variations reported in chromosome number or the difference in opinion on the morphological type of the X-Y pair. [J.N.W.]

The inheritance of paralysed hind legs, scrotal hernia and atresia ani in pigs. S. BEUGS (1941) *J. Heredity* 32, 271

WRIEDT reported the appearance of a recessive sub-lethal factor in a herd of Norwegian Landrace swine. Piglings born to heterozygous matings were 25 per cent affected. Symptoms were paralysed hind legs, the animal being unable to walk, although it could pull itself along with the forelegs. Koroveckaja reported a similar anomaly in Russia, but the proportion of affected piglings was 19.5 per cent, which is less than would be expected of a monogenic recessive factor. It is also reported that in certain other cases the forelegs were involved in individuals of the Large White breed in Sweden and the German Landrace. The forelegs were either bent or greatly enlarged.

Warwick suggested that scrotal hernia in pigs was due to a digenic recessive, and Koroveckaja reported 57.1 per cent males in those litters in which the character was found. The author believes that the factor shows incomplete dominance and points out that, whereas the proportion of males in 414 litters at the Pig Breeding Station, Aas, Norway was 46.9 per cent, the proportion of males in those 31 litters in which scrotal hernia was manifest was 57.14 per cent. The excess of males is obviously caused by the fact that litters with a large number of males have a greater probability to reveal one or more cases of hernia, he says, and confirms a dominant umbilical hernia in the pig reported by Wriedt. Cases of atresia ani are referred to. Both sexes are

involved; the males usually dying after about two or three days of age due to obstruction, the females generally having an opening from the ventral side of the rectum to the sheath which prevents obstruction. Some sows live to maturity and breed. The mode of inheritance of the character is irregular. It was found to be dominant in many cases. Two boars, Jarl and Frick, had 21.2 and 15.3 per cent affected pigs in their litters respectively. Males and females were equally affected. Two sisters bred to Frick, their father, gave 16 per cent affected offspring, but bred to Jarl they gave 26.7 per cent. The author contends that this shows that a monogenic mode of inheritance is out of the question, but he is unable to list the factors involved. Practically, it is impossible to purchase pigs for breeding purposes that one might be certain are free of the genes, causing this character. [J.N.W.]

Genetics of the fowl. 15. Multiple spurs, a mutation linked with duplex comb, F. B. HUNT (1941). *J. Heredity* 32, 357

The modern Black Sumatra cock must have at least three spurs, according to the American Poultry Club. This breed of chickens dates back in the United States to importations in 1847. Females have no spurs. In appearance this breed looks much like game fowls, of which certain ones, said to have originated in India, may have as many as five spurs.

At hatching time there is no difference between the male and female. In adult life the female shows only several enlarged scales at the spur site. Young males show gradual development of several spurs each of which develops much as one would on a single-spurred breed. In the adult male the top spur is generally smaller than the second or largest. Those below are smaller in a descending order. Those which are larger have a separate bony core. Although sexing on the basis of scale appearance at the spur site in the new born chick is not possible, 93 to 98 per cent accuracy is obtained at this age when selecting those that will have multiple spurs from those which will have only a single spur.

During a six year period, involving F_1 , F_2 and back cross population of a Black Sumatra male and Single Comb White Leghorn females, all the F_1 75 per cent, of the F_2 and 50 per cent, of the backcrosses showed multiple spurs. This indicated a unifactorial dominant character. The symbol M was assigned to the gene. Evidence, however, from the progeny of one Single Comb Ancona cock, mated to four Mm hens, to test for the linkage of M , showed that there was, sometimes, a factor that suppressed M , in this case in about 46 per cent of his Mm progeny.

Tests for linkage showed that M does not belong to either of the four autosomal linkage groups previously known, but that it is linked with D , which causes duplex comb. The finding of this relationship established a fifth linkage group. There was some suppression of D noted in this study, but there was no indication that the suppression of D was associated with the suppression of M . [J.N.W.]

Hydrocephalus, a lethal in cattle. C. L. COLLE and L. A. MOORE (1942). *J. Agri. Res.* 65, 483.

Born internal and external types of hydrocephalus have been reported in most animals including man. The condition has sometimes been thought to be lethal in swine and mice; various degrees of association between hereditary and/or lethal hydrocephalus and

flexed tail have been noted in these two animals. Slight hydrocephalus with agnathia in Jersey calves and hydrocephalus in crossbred dogs have also been reported.

Two abnormal calves were born in a grade and purebred Holstein-Friesian herd in the year of 1939-40. The owner suspected his ration, although it was adequate. Examination of the herd showed that the two calves were from sire-daughter matings. By special arrangement 27 additional calves were produced in the herd of such matings. Three peculiarities were noted in these calves: viz. (1) a lethal, (2) asymmetrical faces, and (3) a 'jumpy' nervous condition.

The lethal was manifest by an internal hydrocephalus and marked papilloedema; the skull was enlarged to two or three times its normal size. There were no apparent points of blockage of foramina, unless a very abnormal position of the foramen magnum might have partially or fully blocked the foramina of Magendie and Luschka, thereby causing the hydrocephalus as a secondary manifestation of a gene for bony abnormalities. The humeri and femurs were badly distorted, giving rise to tremendous width of the hips and crooked or twisted forelegs. Asymmetry was shown by a twisted appearance of the face caused not only by the peculiar manner in which the face and head were held, but also by irregularity in the length of the two maxillae and of the two mandibles. In the 'jumpy' condition, muscular co-ordination was lacking, the calf could not stand by itself unsupported, and tremor was present whether standing or lying.

The three characters, it is suggested, are due to three recessive autosomal genes. The symbols given are as follows: normal *L*, lethal *l*, symmetrical *A*, asymmetrical *a*; and normal nervous reaction, *J*, 'jumpy' *j*. On this basis the sire was *LlAaJj*. Daughters of this bull and normal dams would have had eight different genotypes, all normal, but nearly all carriers of one or more of the three recessive genes of the sire. The three abnormal characters would be expected to appear in a definite proportion of the progeny of these daughters and their own sire. The expected and observed distribution of phenotypes in the 27 calves were sufficiently close to confirm the postulated mode of inheritance whether linked or unlinked, although the authors advise further study of the mode of inheritance of 'jumpy' and asymmetry. [J. N. W.]

Sex-linked albinism in the Turkey Meleagris gallopavo. F. B. HUTT AND C. D. MUELLER. *J. Heredity* 33, 69.

STUDIES of an albino poult and its relatives showed that the condition is caused by a sex-linked recessive gene which induces imperfect albinism.

A dingy white characterizes newly hatched affected poult in which the irregular stripes and colour pattern of Bronze poult are faintly visible. Mature birds had some pigment in the feathers which was more evident in the areas normally black. Newly hatched poult have a pale-grey iris which darkens with age. The pupil is red. Histological examination of prepared eye tissue showed absence of melanin in the pecten, the pigment epithelium of the functional retina and the choroid coat where it is normally abundant. Blindness also characterized live affected poult.

In one pen of turkeys where this character first appeared there were 133 normal to 31 albinotic poult in three hatches; this ratio of practically 3:1 suggested a

simple recessive mutation. If it were autosomal, a ratio of 101:34 would have been expected from the 11 hens that produced albinos. The actual ratio was 106:29. If the character were sex-linked the expected ratio of normal to albinotic poult among the 164 offspring from the one male would have been 123:41. The observed ratio fits both expected ratios fairly well.

Eggs from a pen of turkeys among which the character was observed were hatched. Three Bronze coloured toms from these eggs were mated to normal hens and two pens of normal coloured stock were made up from the original pen. Dead embryos were examined: if 24 days old. All albinotic progeny from these matings were female in the ratio of 184 coloured:45 albinos. This again fits the expected ratio, showing that the character is a sex-linked recessive mutation. The symbol *al* is tentatively assigned to the character.

Of the 184 coloured poult from the five carrier toms, 37 died at 24-25 days incubation: of the 45 albinos, 34 had died during this period. The fact that the sex ratio of coloured embryos which died was close to 1 female to 2 male, which is what would be expected, showed that death in the albinos was due to this character and not to the fact they were all female. Differential mortality prior to 24 days may have accounted for the slight difference between the observed and the expected ratios.

With very great difficulty one of the albinotic females which was born alive was raised to maturity. The character reduces the viability of embryos and poult. In the latter case blindness reduces the ability both to find food and of defence. The gene *al*, therefore, can be properly considered as a lethal gene. [J. N. W.]

The talpid lethal in the domestic fowl *R. K. COLE* (1942). *J. Heredity* 33, 83

Low hatchability may sometimes result from the presence of lethal genes which produce visible abnormalities in the embryos. A system of recording the results of examinations of dead embryos, including the age at death, made it possible to recognize the talpid lethal. The name of this lethal was taken from *talpa*, the Latin for mole, because of the shape of the embryonic appendages at death.

The lethal was first found in 1936 when a sire was mated to his own daughters. The system of examining dead embryos revealed the character by the physical abnormalities resulting from it. Having found the lethal it was learned that it had also appeared in 1935 as a result of matings of the same sire to some of his own daughters. Dead embryos due to this gene, were found also in 1937 in eggs from matings of birds distantly related to the sire mentioned. In the latter year only 46.2 per cent of 26 fertile eggs from one hen hatched, not entirely due to this lethal however, whereas 100 per cent of 12 fertile eggs from a full sister hatched. The first common ancestor of all carriers, pedigrees revealed, was a cock hatched in 1927.

The talpid embryos are characterised by reduced length of proximal leg and wing bones, duplication of the distal appendage bones, fused digits in the feet and wings forming what appears like a webbed hand, shortened vertebral column, extreme ectopia and subcutaneous oedema, and a reduction of feather papillae development. Death usually occurs on the 8th to 10th day, although as late as the 17th day, of incubation. Birds heterozygous for the character show none of the abnormalities.

The symbol *ta* is suggested for this simple autosomal recessive character.

Mortality under 4½ days was not classified. Hence there is a slight excess of normal over talpid embryos among those matings where 95 per cent or more of the embryos could be classified. Actually 33·6 per cent of the 415 eggs from such matings produced talpid embryos, whereas 25 per cent would be expected of a simple autosomal recessive. The fewer the deaths under 4½ days the nearer the agreement between the observed and expected incidence of the results of the lethal gene.

Linkage tests were made by examining the linkage of *ta* with three of the five known autosomal linkage groups described by Hutt and Lamoreux. The results were as follows: 1. If *ta* is in the *Cp.R.U.* linkage group, it is not closely linked with *r*; 2. The character *ta* probably does not belong to the *DM* linkage group; and 3.there has been a ratio of parental to non-parental colours of 83 to 93, as compared to the expected ratio of 88 to 88 if *I* and *ta* were not linked in the *P* / *Cr* linkage group; this difference is insignificant. [J. N. W.]

A new type of autosomal nakedness in the domestic fowl, P. D. STURKIE (1942). *J. Heredity* 33, 202

NAKEDNESS in birds is of two types, viz. that in which feather follicles are abnormal, although pterylosis occurs and that in which pterylosis are absent. The abnormality discussed here is of the latter type. The expression of the character varies from almost normal to almost complete nakedness, with degrees of the latter being rated as grades I, II, III and IV, indicating increasing orders of denudation. The frequency with which the different grades of nakedness were observed to occur was in the order II, III, IV, I.

Progeny of a naked male and several heterozygous sons crossed with normal hens were naked and normal in very nearly a 1:1 ratio indicating a simple dominant character. Crosses of *F*₁ naked females with a normal male gave naked males and naked females in a ratio of 1:1, indicating a simple autosomal character. It is believed that the mutation had occurred only recently in a female since the character was observed in only one bird, a male, in 5000 which were progeny of approximately 250 females and 20 males. Its occurrence originally in a male would have resulted, it is thought, in more individuals being involved.

The hatchability of naked and normal chicks was the same. In all other respects, except for feathering, naked chicks were quite normal. There was a high mortality among naked chicks, however, from 10-15 days of age and thereafter. The feeding of wet mash seemed to reduce the death rate in naked chicks so long as it was fed. When dry mash was substituted the death rate would increase. The degree of denudation was not casually related to death rate. It seems, therefore, that this character is at least partially lethal. [J.N.W.]

A new type of recessive achondroplasia in cattle. P. W. GREGORY, S. W. MEAD and W. M. REGAN (1942). *J. Heredity* 33, 317

THE appearance of a sub-lethal hereditary defect, an achondroplastic or bull-dog type, occurred in the Jersey herd of the California Experiment Station. Other authors have reported different types of this defect, of

which one is due to a dominant gene, lethal when homozygous and another is due to a simple recessive gene. Both axial and appendicular skeletal structures are affected as indicated by a short head, deformed jaws, a cleft palate and short stumpy legs. The type reported herein is caused by a monofactorial autosomal recessive.

The defect is variable, is usually lethal and may affect both axial and appendicular skeletal structures. Symptoms in different cases vary from those having a short, broad head, legs of slightly reduced length and a slightly undersized body to those showing such extremely abnormal conditions as a short, broad head, short ears, very irregular incisor teeth in a very short and therefore deformed lower jaw so that the incisors meet the upper jaw opposite the first molars, an extreme degree of cleft palate, a hard palate developed only about half an inch on each side and marked flexion of the anterior pasterns.

It is concluded that the defect is caused by a monofactorial autosomal recessive gene, because the observed incidence fits the expected incidence quite well in each of three types of matings in this herd. These three types of matings were: (1) the mating of a carrier bull to his own daughters or the daughters of another carrier bull.....; (2) the mating of a carrier bull to daughters and great granddaughters of carrier sires..... and (3) the mating of a carrier bull to daughters, grand daughters and great granddaughters of carrier sires.....

One bull, number 320A, was the sire, grand sire or great-grand sire of all affected calves borne, although this bull and one cow, number 344, apparently obtained the gene from their common sire, number 3,000. It is unknown, however, whether the mutation occurred with bull number 3,000 or was handed down to him by or through one parent. This type of achondroplasia seems to differ from a dominant type found in Dexter cattle, but seems to resemble the recessive type first reported in Telemark cattle of Norway. These two latter types do not belong to an allelic series, but involve two independent loci. [J.N.W.]

Bacteriological methods for the large scale detection of Mastitis. G. Davis (1942) *Vet. Rec.* 15, 54

The author emphasises the importance of milk production and the prevention of disease, which decrease milk yield. He lays great stress upon the detection of mastitis and its early diagnosis in large dairy herds. The technique which he advocates for its diagnosis and eradication or control is simple and economic. He has no confidence in any method other than planting freshly taken samples in a non-inhibitory medium and picking off and confirming colonies. Organisms other than *Str. agalactiae* must not be ignored such as *Str. dysgalactiae*, *Str. uberis*, *Str. pyogenes*, *staphylococci* and *micrococci*. Therefore, it is essential that a non-inhibitory medium should be used. Hemolysis alone is not a safe method for the detection of organism carrying mastitis.

He gives in detail the technique to be followed: its outline is, that, afternoon samples are taken aseptically and removed to the laboratory where they are plated on blood or bromocresol purple lactose chalk agar and a resazurin remmet test performed. After incubation of the plates, colonies are transferred to yeast litmus milk, the cultures incubated and inspected and a catalase test carried out. The advantages of the method lie in that centrifuging and the use of blood are unnecessary.* [W.H.D.]

*A minimum of time, labour and apparatus is required.

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